ECOLOGY AND EVOLUTION OF THE DILUTION EFFECT: INTERACTIONS AMONG HOSTS, PARASITES, AND DILUTERS

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Alexander T. Strauss

ECOLOGY AND EVOLUTION OF THE DILUTION EFFECT: INTERACTIONS AMONG HOSTS, PARASITES, AND DILUTERS

The dilution effect is an intriguing, emerging pattern in the community ecology of disease. This pattern links losses of species diversity with elevated disease risk across a wide variety of human, wildlife, and plant disease systems. However, the dilution effect remains controversial. In most cases, it is unclear which 'diluter' taxa drive the pattern, when and how they reduce disease, and why disease dilution can depend on the metric of disease being considered (e.g., infection prevalence vs. density of infected hosts). Here, I develop a predictive, mechanistic framework for the dilution effect in a zooplankton-fungus model system. I uncover which diluters drive this pattern, how and when they reduce disease, and how different mechanisms reduce each metric of disease.

In chapter one, I detect a correlation between diversity and disease in nature, but reveal that this pattern is driven by a key diluter taxa. The focal host here and throughout, *Daphnia dentifera*, is a dominant planktonic grazer in many North American freshwater lakes. It often experiences autumnal epidemics caused by the virulent fungus *Metschnikowia bicuspidata*. Epidemics are smaller in lakes with higher zooplankton diversity, supporting a dilution effect pattern. However, path models reveal that one key diluter taxa (i.e., "small spore predators"), *Ceriodaphnia sp.*, drives this pattern by biasing the index of diversity. Furthermore, these key diluters strongly reduce disease themselves, even though their impacts are embedded within a complex food web. Thus, these diluters drive the dilution effect pattern in nature, especially in lakes with smaller refuges, more intense fish predation, and fewer insect predators.

In chapter two, I bring these key competitor/diluters into the laboratory and test whether they reduce the size of experimental epidemics in focal hosts. At the local scale, these competitor/diluters could reduce disease by consuming parasites (reducing encounters between focal hosts and parasites, without becoming infected), or competing with focal hosts for resources (lowering focal host density, and hence inhibiting density-dependent disease transmission). In a multi-generational mesocosm experiment, presence of competitor/diluters successfully reduces disease. However, in two additional case studies, the dilution effect fails and becomes irrelevant. Parameterized mechanistic models suggest that variation in focal hosts traits drives these divergent outcomes. Thus, while diluters can reduce disease at the local scale, their impacts are not guaranteed to support a dilution effect.

In chapter three, I predict variation among these experimental outcomes from two focal host traits: competitive ability and disease risk. In a second mesocosm experiment, the strength of dilution (i.e., magnitude of reduced disease) is strongest for focal hosts with higher disease risk. However, diluters' reduction of disease fades as focal hosts become more resistant. Disease dilution is also strongest for focal hosts that compete more weakly, since competitor/diluters become more numerous. Finally, path models reveal that diluters' consumption of parasites reduces infection prevalence, but competition with focal hosts reduces the density of infected hosts. Thus, this framework, centered on variation in focal host traits, predicts how and when diluters reduce each metric of disease (infection prevalence vs. density of infected hosts).

Finally, in chapter four, I grapple with the dangers of competition and disease for focal hosts interacting with competitor/diluters and parasites. In an eco-evolutionary mesocosm experiment, the combination of competition and disease dramatically lowers density of focal hosts, despite benefits of disease dilution. Nevertheless, rapid evolution

of higher competitive ability in diverse populations of focal hosts buffers their densities from these negative impacts of competition and disease. Epidemics even accelerate this evolutionary response. However, while these rapidly evolving host populations maintain higher overall densities, they also maintain higher densities of infected hosts (especially when competitor/diluters are absent). Thus, rapid evolution of focal hosts can fundamentally alter costs and benefits of local interactions among focal hosts, parasites, and competitor/diluters.

Although the dilution effect may remain controversial, my dissertation delineates several paths forward. I uncover which diluters drive a dilution effect pattern in nature, and emphasize the need to identify key diluter taxa in other disease systems. I discover how and when diluters reduce disease, and highlight the importance of focal host traits in regulating outcomes of the dilution effect. I reveal that different mechanisms can reduce infection prevalence and density of infected hosts, and stress the importance of a mechanistic framework for predicting these outcomes. Finally, I introduce rapid host evolution as an eco-evolutionary frontier of dilution effect research. Together, these four chapters develop and test a mechanistic framework for the dilution effect. This framework greatly increases power of the dilution effect paradigm.

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Chapter 1

Habitat, predators, and hosts regulate disease in *Daphnia* through direct and indirect pathways

Citation:

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CHAPTER 1 ABSTRACT

Community ecology can link habitat to disease via interactions among habitat, focal hosts, other hosts, their parasites, and predators. However, complicated food web interactions (i.e., trophic interactions among predators, and their impacts on host density and diversity) often obscure the important pathways regulating disease. Here, we disentangle community drivers in a case study of planktonic disease, using a two-step approach.

In step one, we tested univariate field patterns linking community interactions to two disease metrics. Density of focal hosts *(Daphnia dentifera*) was related to density but not prevalence of fungal (*Metschnikowia bicuspidata*) infections. Both disease metrics appeared to be driven by selective predators that cull infected hosts (fish, e.g. *Lepomis macrochirus*), sloppy predators that spread parasites while feeding (midges, *Chaoborus punctipennis*), and spore predators that reduce contact between focal hosts and parasites (other zooplankton, especially small-bodied *Ceriodaphnia sp.*). Host diversity also negatively correlated with disease, suggesting a dilution effect. However, several of these univariate patterns are initially misleading, due to confounding ecological links among habitat, predators, host density, and host diversity.

In step two, path models uncovered and explained these misleading patterns, and grounded them in habitat structure (refuge size). First, rather than directly reducing infection prevalence, fish predation drove disease indirectly through changes in density of midges and frequency of small spore predators (which became more frequent in lakes with small refuges). Second, small spore predators drove the two disease metrics through fundamentally different pathways: They directly reduced infection prevalence, but indirectly reduced density of infected hosts by lowering density of focal hosts (likely via competition). Third, the univariate diversity-disease pattern (signaling a dilution

effect) merely reflected the confounding direct effects of these small spore predators. Diversity *per se* had no effect on disease, after accounting for the links between small spore predators, diversity, and infection prevalence. In turn, these small spore predators were regulated by both size-selective fish predation and refuge size. Thus, path models not only explain each of these surprising results, but also trace their origins back to habitat structure.

INTRODUCTION

Habitat change can increase disease outbreaks (Williams et al. 2002, Patz et al. 2004). Community ecology can explain this connection by linking habitat to disease via variation in density of focal hosts and interactions among them, other hosts, their parasites, and predators (Ostfeld et al. 2008, Johnson et al. 2015). High host density can promote density-dependent disease transmission (Anderson and May 1981). Additionally, predators can drive disease by selectively culling infected hosts (Packer et al. 2003), spreading (Cáceres et al. 2009) or consuming free-living parasites (Johnson et al. 2010), or via other mechanisms less relevant here, including consumption of intermediate hosts for trophically-transmitted parasites (see Johnson et al. 2010). Furthermore, interactions among hosts can also regulate disease transmission (Holt et al. 2003). In the 'dilution effect' paradigm, higher host diversity (specifically, higher frequencies of low competency 'diluter' hosts) reduces disease, because these rarer 'diluters' interfere with disease transmission among more common, more competent focal hosts (Ostfeld and Keesing 2000b, Civitello et al. 2015a). In turn, habitat structure can regulate disease by changing each of these, i.e., through variation in host density (e.g., white nose syndrome in bats: Langwig et al. 2012), changes in predation (amphibian trematodes: Johnson and Chase 2004, schistosomiasis: Sokolow et al. 2015) or abundance of 'diluter' hosts, and hence host diversity (Lyme disease: Ostfeld and Keesing 2000b, Wood and Lafferty 2013). In these examples, links between habitat, density of focal hosts, predation, and diversity of all hosts can pinpoint *why* disease varies among habitats. Thus, these community links provide essential insights for understanding, predicting, or even managing disease across many important systems.

Unfortunately, complicated food web interactions often obscure the important pathways linking habitat to disease. For instance, habitat structure can simultaneously regulate densities of important predators and hosts (Ostfeld et al. 1996, Orrock et al. 2011, Penczykowski et al. 2014). Thus, apparent effects of predators, focal host density, and host diversity can become correlated. Furthermore, interactions among predators and hosts can entangle direct effects on disease with indirect effects. For example, predators can consume each other (Levi et al. 2012, Rohr et al. 2015), lower focal host density (Lafferty 2004, Strauss et al. 2015), change the relative frequencies of high and low competency hosts (Borer et al. 2009), or act as more resistant hosts themselves, hence increasing diversity (Hall et al. 2010, Rohr et al. 2015). Indirect effects of predators, mediated by consumption of other key predators or hosts, can even matter more than their direct influence on disease (e.g., Borer et al. 2009). Disentangling these interactions becomes even more challenging when they depend sensitively on the metric of disease considered. For example, density of infected hosts or vectors (measurements of parasite success) may depend most sensitively on drivers that regulate overall host (or vector) density. In contrast, infection prevalence (a measurement of infection risk) may depend more on drivers that directly interfere with transmission, regardless of host density (e.g., Vanbuskirk and Ostfeld 1995, Randolph and Dobson 2012, Strauss et al. 2015). All of these complications pose major challenges for community ecologists seeking to link habitat to disease using field data.

Path models firmly grounded in natural history can provide a solution to these problems (see Grace et al. 2010). Here, we illustrate a two-step approach in a case study of planktonic disease (see Hall et al. 2010). In **step one**, we identify theoretically relevant drivers of disease and their interactions, and test all relationships with univariate field patterns. We begin by introducing our study system and the role of focal host density as a potential disease driver. Then, we review and test three general and

relevant modes of predation on disease (Table 1). Next, we describe and test six types of complicating but essential links among habitat structure, host density, predators, and host diversity. Specifically, Links 1-4) predators can be regulated by habitat structure and other predators, and Link 5) density of focal hosts and Link 6) host diversity can both be regulated by predators. In turn, host diversity also appears linked to disease. In **step two**, the univariately significant ecological links guide the creation of path models. Path models disentangle direct effects of predators from their indirect effects on disease, and distinguish spurious correlations from causal drivers. We fit separate path models to predict infection prevalence and then density of infected hosts. These separate models highlight key differences among the strengths of links (paths) from habitat to these disease metrics. With this two-step approach, we uncover the most important species interactions driving disease, and ground them in habitat structure.

STEP ONE – THEORETICALLY RELEVANT DRIVERS AND LINKS (UNIVARIATE)

Study system

Focal host and parasite

Our focal host, the cladoceran zooplankter *Daphnia dentifera*, is a dominant, non-selective grazer in many freshwater lakes in North America (Tessier and Woodruff 2002), including the southwestern Indiana lakes studied here. In many lakes, this host experiences autumnal epidemics of a virulent fungus, *Metschnikowia bicuspidata* (Overholt et al. 2012, Penczykowski et al. 2014). Hosts encounter infectious fungal spores while non-selectively filter-feeding for algal food (Hall et al. 2007). Infected hosts cannot recover and die from infection. After host death, spores are released back into the water column. Thus, *M. bicuspidata* acts as a parasitic obligate killer (Ebert and Weisser 1997). With this natural history, transmission could increase with higher **host density** and higher density of free-living fungal spores (Anderson and May 1981).

Three Modes of Predation

Three modes of predation appear to regulate fungal epidemics in lake populations of our focal host. Each mode is grounded in general theory and arises in other host-parasite systems (Table 1). First, s**elective predators** (bluegill sunfish [*Lepomis macrochirus*]) selectively target and cull infected hosts, reducing prevalence and density of infections (Packer et al. 2003, Hall et al. 2005; the 'healthy herds' hypothesis). Fungal infection makes hosts opaque, and hence more conspicuous to fish predators (Duffy and Hall 2008). Fish then consume parasites along with infected hosts ("concomitant predation"; see Johnson et al. 2010), resulting in a net loss of fungal spores. Thus, high fish predation lowers infection prevalence of focal hosts (Hall et al. 2005, Hall et al. 2010).

Second, **"sloppy" predators** (*Chaoborus punctipennis* midge larvae) distribute infectious spores when they attack infected prey. Midge predators release spores higher in the water column, alleviating an environmental trap created when dead infected hosts sink. Focal hosts consume these dispersed spores, *increasing* infection prevalence (Cáceres et al. 2009). Midges can also induce changes in host phenotype that increase susceptibility (Duffy et al. 2011). High midge density correlates with higher infection prevalence in two sets of lakes (Hall et al. 2010, Penczykowski et al. 2014). Thus, selective and sloppy predators have opposite effects on disease spread.

Third, **spore predators** (other non-selective zooplankton [cladoceran] filter-feeders) consume free-living parasites while rarely becoming sick. Spore predation reduces contact between focal hosts and parasites (Johnson et al. 2010). In our study system, spore predators can also compete with focal hosts, and contribute to host diversity (see more below). The most common spore predator taxa in our lakes (*Ceriodaphnia sp.*) highly resists infection, and the second most common (*D. pulicaria*) is

almost completely immune. The former can reduce prevalence and density of infections in experiments, and both appear to reduce infection prevalence in lake communities (*D. pulicaria*: Hall et al. 2009, *Ceriodaphnia*: Strauss et al. 2015). Other even rarer cladoceran spore predators co-occur, but they rarely (if ever) become infected in lakes we sample (SRH, unpublished). Thus, these three modes of predation (selective, sloppy, and spore predation) each regulate disease through distinct mechanisms.

Links 1-4): Predators may be regulated by habitat structure and other predators

Refuge size, a critical habitat variable, varies among lakes and regulates selective fish predation. Visually oriented fish predators target large, conspicuous zooplankton (Brooks and Dodson 1965, Vanni 1986). However, large zooplankton can escape fish predation in the deep water refuge habitat. This refuge habitat is bounded at the top by temperature change (due to habitat choice by warm-water fishes), and at the bottom by oxygen depletion (due to physiological demands of zooplankton). Intensity of fish predation proves difficult to measure directly, but small body size of focal hosts indicates more intense predation (e.g., Mills and Schiavone 1982, Vanni 1986, Carpenter et al. 1987). Thus, smaller refuges should cause more intense fish predation (i.e., smaller focal host body size; **Link 1**).

Trophic interactions among predators, regulated by refuge size, could confound direct (Table 1) and indirect drivers of disease. Fish predators consume sloppy midge predators, and midge predators can also seek deep water refuge from fish predation (Gonzalez and Tessier 1997). Thus, intensity of fish predation (**Link 2a**) and/or refuge size (**Link 2b**) could regulate the density of midge predators. Furthermore, midges are gape-limited, preferentially culling smaller hosts (Pastorok 1981), and can induce plastic increases in host body size (Duffy et al. 2011). Thus, midges could also potentially

Table 1. Three modes of predation and their direct effects on disease: general theory, empirical examples, and natural history in the study system here, with a zooplankton focal host (*Daphnia dentifera*) and a fungal parasite (*Metschnikowia bicuspidata*).

Predation Mode & General Theory	**Select Empirical Examples**	***Daphnia* / *Metschnikowia* system**
Selective Predation ***Theory:*** Selective predators target and cull infected prey, reducing prevalence, density, or intensity of infections (Hudson et al. 1992, Packer et al. 2003, Hall et al. 2005).	• Selective prawn predators target schistosome-infected snails, and appear to reduce schistosomiasis transmission (Sokolow et al. 2015). • Selective piscivorous fish target lice-infected juvenile salmon, likely lowering sea lice infection loads (Krkosek et al. 2011). • Selective spiders target fungus-infected grasshoppers, reducing parasite-driven host mortality (Laws et al. 2009). • Selective wolves appear to target moose heavily infected with tapeworms, reducing infection burdens (Joly and Messier 2004). • Selective foxes appear to target heavily infected grouse, potentially lowering nematode infection burdens (Hudson et al. 1992).	Bluegill sunfish (*Lepomis macrochirus*) predators target infected hosts because fungal infection make hosts conspicuous (Duffy and Hall 2008). Selective fish predation appears to lower infection prevalence (Hall et al. 2010).
Sloppy Predation ***Theory:*** Sloppy predators (or herbivores, or	• Sloppy *Didinium* predators may increase infectious free living bacteria, when attacking infected *Paramecium* prey (Banerji et al. 2015). • Sloppy butterflyfish attack infected coral and enhance water-borne transmission	Larval *Chaoborus* midges regurgitate spores after attacking

scavengers) can distribute infectious free-living parasites when they attack infected prey (Cáceres et al. 2009, Auld et al. 2014).	of black-band disease (Aeby and Santavy 2006). • Sloppy beetle herbivores spread rust fungus spores (potentially long distances) after foraging on infected musk thistle (Kok and Abad 1994). • Sloppy jackal or vulture scavengers may distribute anthrax spores away from ungulate carcasses through feces (Lindeque and Turnbull 1994).	infected hosts (Cáceres et al. 2009). High midge density correlates with high infection prevalence (Hall et al. 2010).
Spore Predation *(more generally:* ***predation of free-living parasites****)* ***Theory:*** Predators of free-living parasites can consume parasites without becoming infected. Spore predation reduces encounters between focal hosts and parasites and can lower infection prevalence or density of infections	• Zooplankton consume free-living chytrid zoospores, potentially suppressing outbreaks of algal chytrids (reviewed: Kagami et al. 2014). • Aquatic micropredators consume fungal zoospores, reducing infection rates of chytridiomycosis in amphibians (Schmeller et al. 2014). • Damselfly nymphs consume free-living trematode larvae, reducing *Ribeiroia* infections in amphibian hosts (Orlofske et al. 2012). • Small fishes consume free-living trematode larvae, potentially reducing transmission success to final hosts (Kaplan et al. 2009). • Predatory fungi capture and consume free-living nematodes, even after passage through dog gastrointestinal tracts, offering potential biocontrol for nematodes infecting mammals (Carvalho et al. 2009).	Cladoceran spore predators inadvertently "vacuum" spores while filter-feeding. They rarely (small *Ceriodaphnia sp.*) or never (large *D. pulicaria*) become infected. Both taxa appear to reduce prevalence and/or density of infections (Hall et al. 2009, Hall et al. 2010,

(Johnson et al. 2010, Strauss et al. 2015).	• Dung beetles feed on parasitic nematodes and protozoans, broadly reducing transmission to livestock, wildlife, and humans (reviewed: Nichols et al. 2008).	Penczykowski et al. 2014, Strauss et al. 2015).

impact the fish predation index (body size of focal hosts). Either way, fish predation intensity and midge density should be negatively correlated.

Both fish predators and midge predators selectively consume spore predators based on body size. Visually oriented fish target larger taxa, while gape-limited midges target smaller taxa (Gonzalez and Tessier 1997, Tessier and Woodruff 2002). The most common spore predator is small, and hence less conspicuous to fish but more susceptible to midges (*Ceriodaphnia;* hereafter: small spore predators. Frequency of these small spore predators within the host community should be higher in lakes with smaller refuges (**Link 3a**), more intense fish predation (**Link 3b**), and fewer midge predators (**Link 3c**). Larger bodied *Daphnia pulicaria* (hereafter: large spore predators) are more vulnerable to fish and less to midges. Moreover, these large spore predators compete superiorly without fish predation (Leibold 1991). Thus, they should become more frequent in lakes with larger refuges (**Link 4a**), less intense fish predation (**Link 4b**), and more midge predators (**Link 4c**). Overall, variation in refuge size and predation regimes should govern the importance of these two spore predators and perhaps restrict them to different types of lakes. All of these trophic interactions create interpretation problems with univariate data, because apparent effects of predators on disease could actually arise from changes in their prey (other predators).

Link 5): Host density may be regulated by predators

When disease transmission is density dependent, species interactions that regulate host density could indirectly drive disease (Anderson and May 1981). For example, predators that consume focal hosts and reduce their density can inhibit disease spread (e.g., Lafferty 2004). Alternatively, competitors can inhibit disease spread if they reduce focal host density by depleting shared resources (e.g., Mitchell et al. 2002). Fish predators and midge predators both consume focal hosts, and spore predators compete with focal hosts for shared algal resources (Gonzalez and Tessier 1997, Tessier and Woodruff 2002, Hall et al. 2009, Strauss et al. 2015). Thus, focal host density could be lower in lakes with more intense fish predation (**Link 5a**) or more midge predators (**Link 5b**), or in lakes dominated by small spore predators/competitors (**Link 5c**) or large spore predator/competitors (**Link 5d**). These potential indirect effects mediated by host density could even exceed the direct effects of these predators on disease (Table1).

Moreover, the importance of density-mediated effects could depend on the disease metric considered. Indirect effects mediated by density of focal hosts depend on strong links between focal host density and disease. However, host density can be more closely linked to density of focal host infections than infection prevalence, for example, due to non-linear density-prevalence relationships (Civitello et al. 2013). Thus, predators that regulate focal host density may primarily drive variation in density of infected hosts. In contrast, predators that interfere with transmission through other mechanisms might more strongly drive variation in infection prevalence (see Vanbuskirk and Ostfeld 1995, Randolph and Dobson 2012, Strauss et al. 2015). Here, spore predators uniquely drive disease through two mechanisms: lowering focal host density via competition, *and* consuming of free-living parasites (Hall et al. 2009, Strauss et al.

2015). Thus, the relative importance of these two mechanisms could depend on the metric of disease considered (prevalence vs. density of infections).

Link 6): Host diversity may be regulated by spore predators (hosts themselves)

The roles of spore predators also become entangled with a potentially spurious 'dilution effect'. A dilution effect associates decreases in **host diversity** with increases in disease risk for a focal host species (Ostfeld and Keesing 2000a, Keesing et al. 2006, Civitello et al. 2015a). This pattern emerges when rarer 'diluters' interfere with transmission among more competent, more common focal hosts. Interference can occur through spore predation (Johnson et al. 2010) or competition with focal hosts (Keesing et al. 2006). Thus, spore predators may serve as potential 'diluters' in our study system. Critically however, a spurious diversity-disease correlation could merely reflect the impacts of certain spore predators reducing disease, rather than any effects of host diversity *per se* (see LoGiudice et al. 2003, Randolph and Dobson 2012). This spurious result could occur if spore predators simultaneously reduce disease and increase our index of host diversity.

Accounting for links between spore predator frequencies and host diversity may help disentangle these potential impacts of host diversity *per se* from impacts of key spore predators. Because host communities in our lakes are so uneven (see below), we represent host diversity (including both focal hosts and spore predators) with the inverse Simpson's diversity index. With focal hosts dominating most of our lake communities, host diversity should increase with higher frequencies of small spore predators (**Link 6a**), large spore predators (**Link 6b**), and other spore predators (**Link 6c**). However, as spore predators become even more frequent and begin to dominate, a higher frequency of spore predators will actually decrease the inverse Simpson's host diversity index. By including a few of these types of lakes, we may be able to decouple host diversity (which

would begin to decline) from frequencies of key spore predators (which would continue to increase). Thus, it may become possible to disentangle direct effects of host diversity from spore predation. In other words, by linking spore predators to host diversity, we can test whether host diversity *per se* drives disease, or whether a spurious dilution pattern arises merely through correlation with key, relatively rare, spore predators.

Study system summary

Three modes of predation—selective, sloppy, and spore—appear relevant to our study system (Table 1). Habitat structure could directly or indirectly regulate all of them, based on decades of natural history research. However, trophic interactions among predators and their effects on host density and diversity could confound direct effects with indirect effects of predators on disease. Altogether, six ecological links obscure the most important pathways linking habitat to disease (see Table 2). Moreover, these most important paths could depend on the disease metric examined. To continue, we must first test each of these potential disease drivers (host density, modes of predation, and host diversity) and each ecological link with univariate field patterns. Then, we can begin to synthesize disease drivers and their interactions with path analysis.

Univariate Analyses

Field Sampling Methods

We sampled lakes in Green and Sullivan counties (Southwest Indiana, USA) during epidemics of focal hosts (mid August – early December). The sampling regime differed slightly among years: we visited 15 lakes in 2010 (visited weekly), 18 in 2009 (weekly), and 28 in 2014 (fortnightly). At each visit we collected two samples of zooplankton, each pooling three vertical tows of a Wisconsin net (13 cm diameter, 153 µm mesh). With the first sample, we measured body size (~ 40+ focal host adults) and

visually screened live focal hosts (400+) for infections. Mean body size of adult hosts provides the index of intensity of fish predation. Infection prevalence was calculated as the proportion of these focal hosts that were infected.

The second sample was preserved to estimate areal densities of focal hosts and midge larvae. We also estimated frequencies of focal hosts (mean frequency: 72%; maximum: 99%) and spore predators within the host (cladoceran) community (small bodied *Ceriodaphnia sp.* [15%, 79%], large *D. pulicaria* [8%, 44%] and all others lumped together [*Bosmina sp.*:3%, 28%; *Diaphanosoma sp.*: 0.7%, 12%; , *D. parvula*: 0.4%, 10%; *Alona sp.* & *Chydorus sp.*: 0.2%,1.4%, and very rare *D. ambigua* and *Scapholebris sp.*]). We calculated inverse Simpson's diversity index of this total host community (focal hosts and all spore predators). Infection prevalence of focal hosts was multiplied by their total areal density to yield density of infected hosts. Finally, we estimated refuge size with vertical casts of a Hydrolab multiprobe, taking temperature and oxygen at every 0.5 to 1.0 m. Refuge size was calculated as the difference between the depth of the thermocline (upper bound, defined as maximum buoyancy frequency) and the oxygen threshold (lower bound, 1 mg/L) (see Penczykowski et al. 2014). For each lake x year combination, we calculated a season (Sep.-Nov.) average for each variable.

Statistical methods

All statistical models were fit using R (R Development Core Team 2010). Predation modes (Table 1) and ecological links (Table 2) were tested individually with univariate mixed effect models in the package NLME (Pinheiro and Bates 2000). 'Lake' was included in all models as a random effect (intercept only). With only three years of data, we modeled 'year' as a fixed (rather than random) effect. With this baseline model structure, we then used likelihood ratios to test significance of each relationship. Density of sloppy midge predators was log transformed prior to analyses. However, all other

data remained untransformed in order to preserve their natural variance structures. We explicitly modeled variance of all response variables with exponential or power functions to describe the heteroskedasticity in the data (see Pinheiro and Bates 2000).

Univariate disease driver results

Field patterns supported host density, all three modes of predation, and host diversity as potential disease drivers. Density of focal hosts was not correlated with infection prevalence (Fig. 1 A; $P = 0.25$). However, it was positively correlated with infected host density (Fig. 1 B; $P < 0.0001$). For all other potential drivers, impacts on infected host density (Fig. S1) qualitatively mirrored those on infection prevalence (Fig. 2). Lakes with more selective fish predation (indexed by body size of focal hosts) had lower prevalence (Fig. 2 A; $P < 0.0005$) and density of infections (Fig. S1 A; $P < 0.0004$). In contrast, lakes with higher densities of sloppy midge predators (*Chaoborus*) had higher prevalence (Fig. 2 B; $P < 0.0001$) and density of infections (Fig. S1 B; $P < 0.0001$). Furthermore, lakes with higher frequencies of small spore predators (*Ceriodaphnia*) and other spore predators had lower prevalence (Fig. 2 C & E; both $P < 0.0005$) and density of infections (Fig. S1 C & E; $P = 0.0024$, $P < 0.0001$, respectively). However, frequency of large spore predators (*D. pulicaria*) was unrelated to prevalence (Fig. 2 D; $P = 0.58$) or density of infections (Fig. S1 D; $P = 0.38$). Finally, high host diversity also correlated with low prevalence (Fig. 2 E; $P = 0.0074$) and density of infections (Fig. S1 E; $P < 0.0005$), consistent with the prediction of a dilution effect.

Univariate ecological link results

Links among habitat structure, predators, host density, and host diversity complicated interpretation of these potential disease drivers (see Table 2 for statistical significance of each link). Smaller refuges from fish marginally (but not significantly)

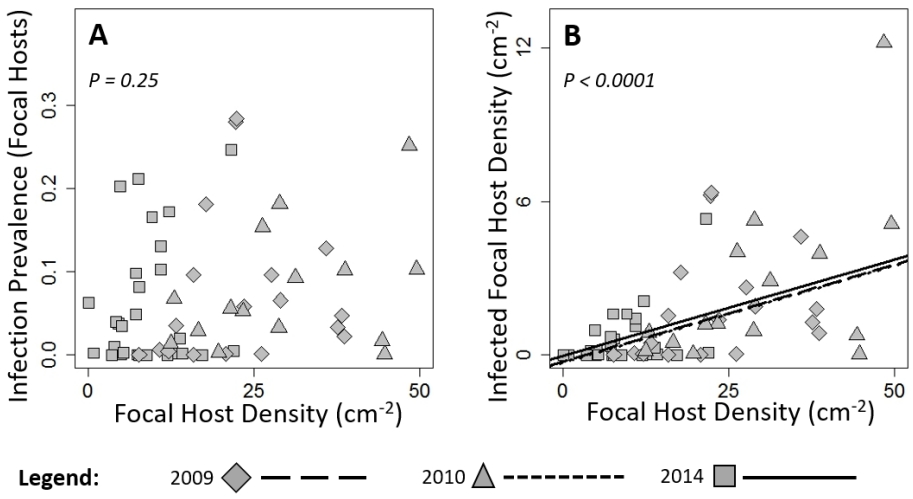

Figure 1. Overall density of focal hosts (*Daphnia dentifera*) **A)** does not drive infection prevalence, but **B)** does drive density of infected focal hosts. Each point is a lake population in a given year (2009, 2010, and 2014). Infection prevalence is mean proportion of focal hosts infected during an epidemic season. Infected host density is mean density of infected focal hosts over the same time period. Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

increased the intensity of fish predation (i.e., decreased body size of focal hosts [Link 1; Fig. 3 A]). However, more intense fish predation did reduce density of sloppy midge predators (Link 2a; Fig. 3 B). In turn, frequency of small spore predators (*Ceriodaphnia*) increased with smaller refuges (Link 3a; Fig. 3 D), more intense size-selective fish predation (Link 3b; Fig. 3 E), and lower densities of gape-limited midges (Link 3c; Fig. 3 F). On the opposite side of the refuge spectrum, frequency of large spore predators (*D. pulicaria*) increased with larger refuges (Link 4a; Fig. 3 G), less intense size-selective fish predation (Link 4b; Fig. 3 H), but lower densities of gape-limited midge predators (opposite of the prediction based on natural history, but only marginally significant; Link 4c; Fig. 3 I). Thus, predators were regulated by habitat structure and each other.

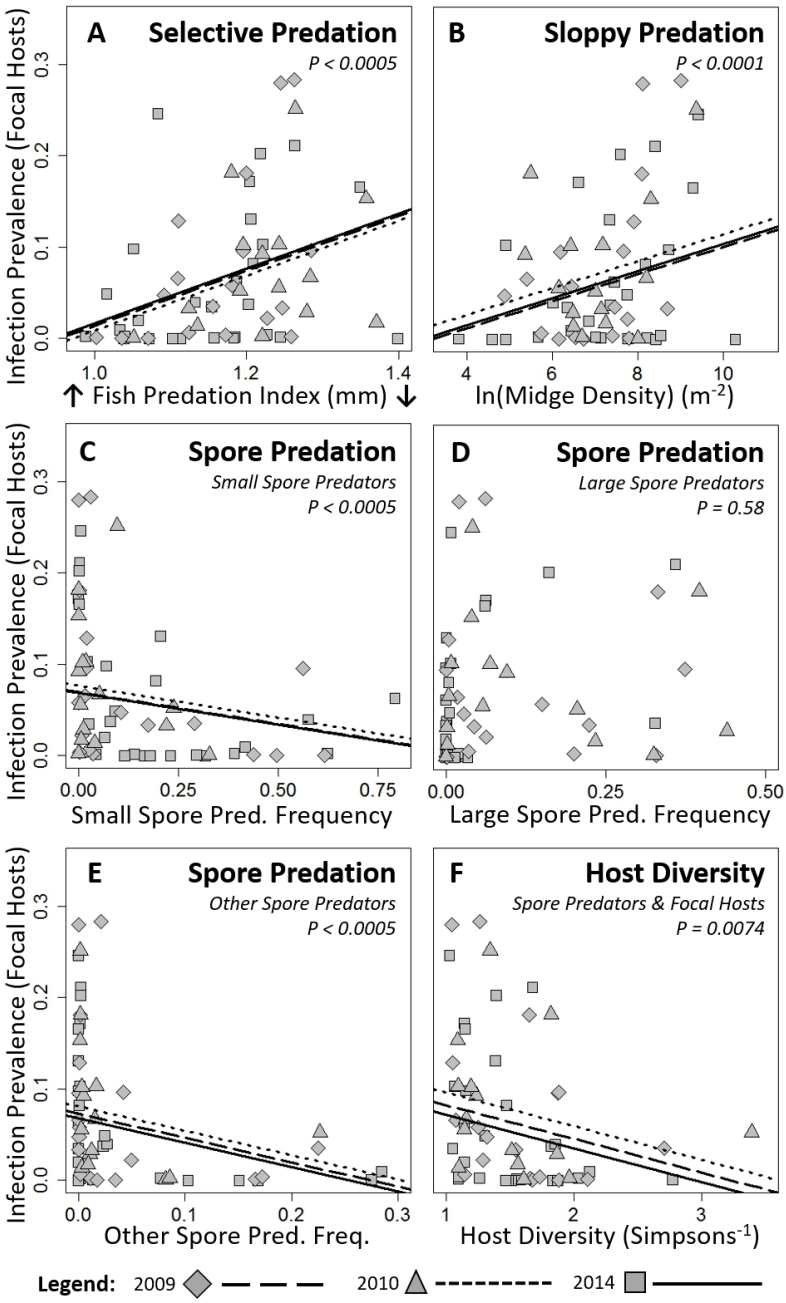

Figure 2. Three modes of predation (Table 1) correlate with infection prevalence of the focal host zooplankton (*Daphnia dentifera*). Infection prevalence is mean proportion of focal hosts infected during an epidemic season. Each point is a lake population in a given year. **A)** *Selective Predation:* Fish predation is indexed by body size of adult focal hosts (mm). Smaller size = more fish predation (↑); larger size = less (↓). More selective fish predation (left on x-axis) correlated with lower infection prevalence. **B)** *Sloppy Predation:* More sloppy midge predators (*Chaoborus*) correlated with higher infection prevalence. **C-E)** *Spore Predation:* **C)** High frequencies within the host community of small spore predators (*Ceriodaphnia*) correlated with lower infection prevalence. **D)** Frequency of large spore predators (*D. pulicaria*) did not, but **E)** frequency of other spore predators also did. *Host Diversity:* Finally, **F)** higher host diversity (focal hosts and spore predators) also correlated with lower infection prevalence, consistent with a dilution effect. Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

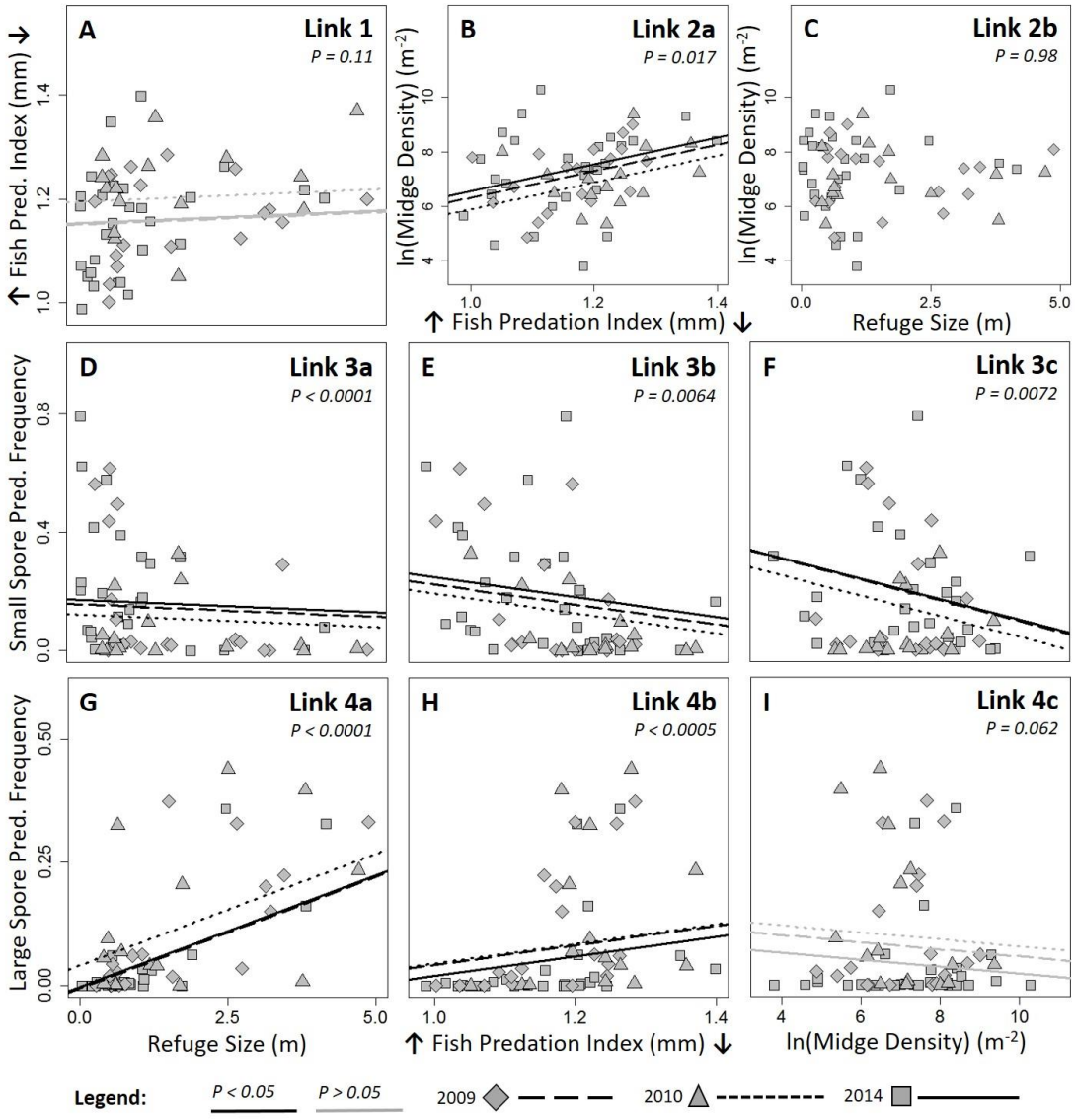

Figure 3. Predators were regulated by habitat structure and trophic interactions with other predators (Links 1-4; see Table 2). Each point is a lake population in a given year. **A)** Small refuge habitats had only marginally more fish predation. **B)** More intense fish predation (smaller adult focal host size; left on x-axis) correlated with fewer sloppy midge predators (*Chaoborus*). However, **C)** refuge size did not predict midge density. Small spore predators were more frequent when **D)** refuge size was smaller, **E)** fish predation intensity was higher, and **F)** midge density was lower. In contrast, large spore predators were more frequent when **G)** refuge size was larger, **H)**, fish predation intensity was lower, and **I)** midge density was lower (marginally). Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

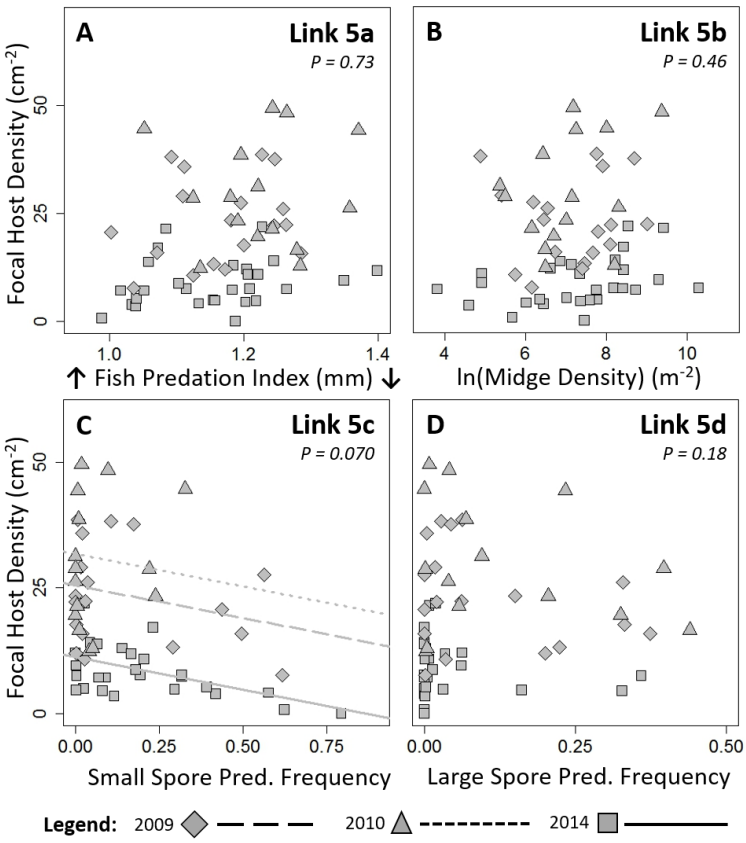

Figure 4. Focal host density (*Daphnia dentifera*) was only marginally regulated by small spore predators (Link 5, see Table 2). Each point is a lake population in a given year. Focal host density was not reduced by **A)** fish predation intensity or **B)** midge predator density (both are predators of focal hosts). **C)** Focal host density was marginally lower in lakes with higher frequencies of small spore predators (*Ceriodaphnia*), but **D)** not in lakes with higher frequencies of large spore predators (*D. pulicaria*) (both spore predators compete with focal hosts). Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

Density of focal hosts was much less responsive to these predators, however. In fact, it only decreased with higher frequency of small spore predators (marginally significant Link 5c; Fig. 4 C, likely due to competition). All other links with densiy of focal hosts were insignificant (Links 5a,b&d corresponding to Fig. 4 A, B & D, respectively). Finally, host diversity increased with higher frequencies of small (Link 6a), large (Link 6b), and other spore predators (Link 6c), since all of them were relatively rare (Fig. 5 A-C, respectively). Thus, density of focal hosts and diversity of host communities (two potential disease drivers) were linked via the community composition of spore predators.

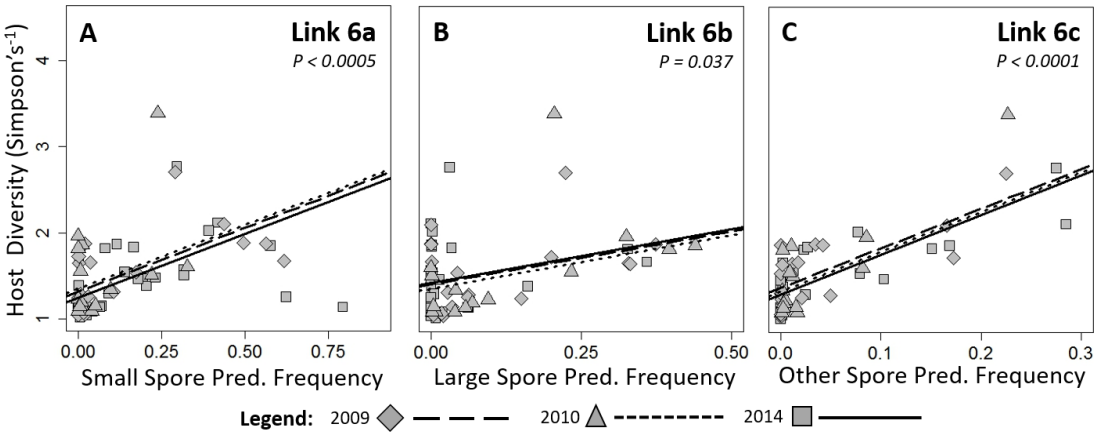

Figure 5. Diversity of the host community (i.e., focal hosts [*Daphnia dentifera*] and spore predators) was strongly regulated by frequency of each group of spore predators. Spore predators are themselves hosts, but are all rarer than focal hosts. Each point is a lake population in a given year. Higher frequencies of **A)** small spore predators (*Ceriodaphnia*), **B)** large spore predators (*D. pulicaria*), and **C)** other spore predators all increased host diversity. Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

This multitude of significant, univariate links (see Table 2) potentially confound disease drivers (Figs. 2 & S1). Hence, we turned to path analysis to disentangle them.

STEP TWO – SYNTHESIZING DISEASE DRIVERS

Path Analysis Methods

To work through these complicated interactions, we used path analysis. To fit path models, we used the package lavaan (Rosseel 2012), weighting observations using the package lavaan.survey (Oberski 2014) to account for non-independence of the same lakes sampled in separate years. Given the limits of our dataset, we tested three complementary models. Model 1 disentangled drivers of infection prevalence, and

model 2 disentangled drivers of density of infected hosts (hence, it includes 'focal host density' [Fig. 1 B]). Unfortunately, we could not include 'host diversity' in model 2, due to collinearity among too many disease drivers. Therefore, in order to more directly compare drivers of prevalence versus density of infections, we fit a third model. Model 3 is nearly identical to model 1, but it also includes 'focal host density' and omits 'host diversity'. These modifications create a parallel structural form for comparison with model 2.

All models were constructed, fit, and assessed using a robust, pre-determined protocol. First, all significant and trending univariate patterns were included in each appropriate path model (excepting the limitations due to collinearity, described above). Two links (between the 'fish predation index' and 'midge density', and between 'small spore predator frequency' and 'focal host density') were fit as covariances, implying correlation. All other links were fit as regressions, implying causality. Additional covariances were included for correlations among frequencies of spore predators (since they shared a common denominator). Second, models were fit with a maximum likelihood estimator (MLM) that was robust to non-normal standard errors and used a robust Satorra-Bentler chi-square test statistic (Satorra and Bentler 2001). After model fitting, residual covariances were inspected in order to identify any potentially missing links. Through this process, the link between refuge size and the index of fish predation (Link 1) was added to all three models. Third, we assessed model fits with several robust criteria, including CFI, TLI, RMSEA, and SRMR test statistics (Hu and Bentler 1999) (see Appendix S1 in Supporting Information for details). Finally, we extracted *P* values and standardized parameter estimates (SPE's) for each relationship. These SPE's were used to compare effect sizes among paths in our final models.

Table 2. Six ecological links among habitat, predators, density of focal hosts, and diversity of the host community complicate disease drivers in the study system with zooplankton focal hosts (*Daphnia dentifera*) and fungal parasites (*Metschnikowia bicuspidata*). Column 1 delineates each link, column 2 reviews relevant natural history theory, and column 3 reports statistical significance as a univariate pattern. Columns 4 and 5 report *P* values and standardized parameter estimates with links as paths in path model 1(disentangling drivers of infection prevalence), and path model 2 (disentangling drivers of density of infected hosts). Ecological links in path models 2 and 3 are quantitatively identical (column 5). Significant and trending *P* values ($P < 0.1$) are bold.

Ecological Link	**Natural History Theory**	**Univariate Result**	**Path Mod. 1 (Fig. 6)**	**Path Mod. 2 & 3 (Fig. 7)**
Link 1: Regulators of Intensity of Selective Predation (Fish, e.g., *Lepomis macrochirus*):	**1)** Prey escape fish predation in the refuge. Small refuges should increase[1]	*P* = 0.11 Fig. 3 A	***P* = 0.004** SPE = 0.297	
Link 2: Regulators of Density of Sloppy Predators (Midge, *Chaoborus punctipennis):*	**2a)** More intense fish predation should decrease (via predation)[2]	***P* = 0.017** Fig. 3 B	***P* = 0.052** SPE = 0.281	
	2b) Larger refuges from fish predation should increase[2]	*P* = 0.98 Fig. 3 C	*Univariate relationship not significant or trending*	
Link 3: Regulators of Frequency of Small Spore	**3a)** Smaller refuges from fish should increase (small = inconspicuous)[2]	***P* < 0.0001** Fig. 3 D	***P* = 0.009** SPE = -0.251	***P* = 0.037** SPE = -0.211

Predators (Zooplankton, *Ceriodaphnia sp*.):	**3b)** More intense fish pred. should increase (small = inconspicuous)[1]	***P* = 0.0064** Fig. 3 E	***P* = 0.002** SPE = -0.351	***P* = 0.09** SPE = -0.358
	3c) Lower gape-limited midge density should increase (small = susceptible)[3]	***P* = 0.0072** Fig. 3 F	*P* = 0.75 SPE = -0.039	*P* = 0.89 SPE = -0.016
Link 4: Regulators of Frequency of Large Spore Predators (Zooplankton, *Daphnia pulicaria*):	**4a)** Larger refuges from fish should increase (large = conspicuous)[4]	***P* < 0.0001** Fig. 3 G	***P* < 0.001** SPE = 0.600	***P* < 0.001** SPE = 0.608
	4b) Less intense fish predation should increase (large = conspicuous)[1]	***P* < 0.0005** Fig. 3 H	***P* = 0.002** SPE = 0.254	***P* = 0.003** SPE = 0.236
	4c) Higher gape-limited midge density should increase (large = resistant)[2]	****P* = 0.062** Fig. 3 I	*P* = 0.30 SPE = -0.075	*P* = 0.35 SPE = -0.070
Link 5: Regulators of Density of Focal Hosts	**5a)** More intense fish predation should decrease (via predation)[2]	*P* = 0.73 Fig. 4 A	*Univariate relationship not significant or trending*	

(Zooplankton, *Daphnia dentifera*):	**5b)** Higher midge density should decrease (via predation)[2]	*P* = 0.46 Fig. 4 B	*Univariate relationship not significant or trending*	
	5c) Higher freq. small spore pred. should decrease (via competition)[4]	***P* = 0.070** Fig. 4 C	*Host density unimportant (Fig. 1 A)*	***P* = 0.070** SPE = -0.240
	5d) Higher freq. large spore pred. should decrease (via competition)[5]	*P* = 0.18 Fig. 4 D	*Univariate relationship not significant or trending*	
Link 6: Regulators of Host Diversity (Zooplankton: Focal Hosts and Spore Predators):	**6a)** Higher freq. small spore pred. should increase (because rare)	***P* < 0.0005** Fig. 5 A	***P* < 0.001** SPE = 0.365	† *collinearity among disease predictors*
	6b) Higher freq. large spore pred. should increase (because rare)	***P* = 0.037** Fig. 5 B	***P* < 0.001** SPE = 0.479	
	6c) Higher freq. rare spore pred. should increase (because rare)	***P* < 0.0001** Fig. 5 C	***P* < 0.001** SPE = 0.664	

* = univariate trend detected in the opposite direction than predicted from theory (Link 4c)

† = links not included, because inclusion of the 'dilution effect' link between diversity and disease created collinearity among disease predictors (path models 2 and 3)

References: [1] (Tessier and Woodruff 2002). [2](Gonzalez and Tessier 1997). [3] (Wissel et al. 2003). [4](Tessier and Welser 1991). [4](Strauss et al. 2015).[5] (Hall et al. 2009).

Path Analysis Results

Fit statistics confirmed good fits of all three path models (see Table S1). Table 2 delineates each ecological link, reviews theory behind the relevant natural history of the plankton system, and reports its statistical significance as a univariate pattern and link inpath models 1, 2, and 3, where applicable (see Tables S2-S4 for parameter estimates and more details).

Path model 1: Disease drivers & underlying ecological links

Path model 1 (Fig. 6) disentangled drivers of infection prevalence (Fig. 2). Lakes with small refuges had more intense fish predation (Link 1), which in turn reduced density of sloppy midge predators (Link 2a). Together, small refuges (Link 3a) and more intense fish predation (Link 3b) increased frequency of small spore predators. In contrast, larger refuges (Link 4a) and less intense fish predation (Link 4b) increased frequency of large spore predators. Even after accounting for these ecological links, high frequency of small spore predators (*Ceriodaphnia*) still directly reduced infection prevalence (P = 0.048; SPE = -0.231). Simultaneously, high density of sloppy midge predators (*Chaoborus*) directly increased infection prevalence (P = 0.026; SPE = 0.294). However, the index of selective fish predation no longer exerted a significant direct effect on infection prevalence (P = 0.47; SPE = 0.098), even though it appeared important univariately (Fig. 2 A). Instead, fish drove indirect effects on disease, mediated trophically through changes in small spore predators and sloppy midge predators. Furthermore, frequency of other spore predators no longer significantly reduced prevalence of infection (P = 0.103; despite the relatively strong effect, SPE = -0.332). Finally, the negative diversity-disease pattern detected univariately (a dilution effect; Fig. 2 F) now disappeared (P = 0.79; SPE = 0.063). Instead, the path model clarified that

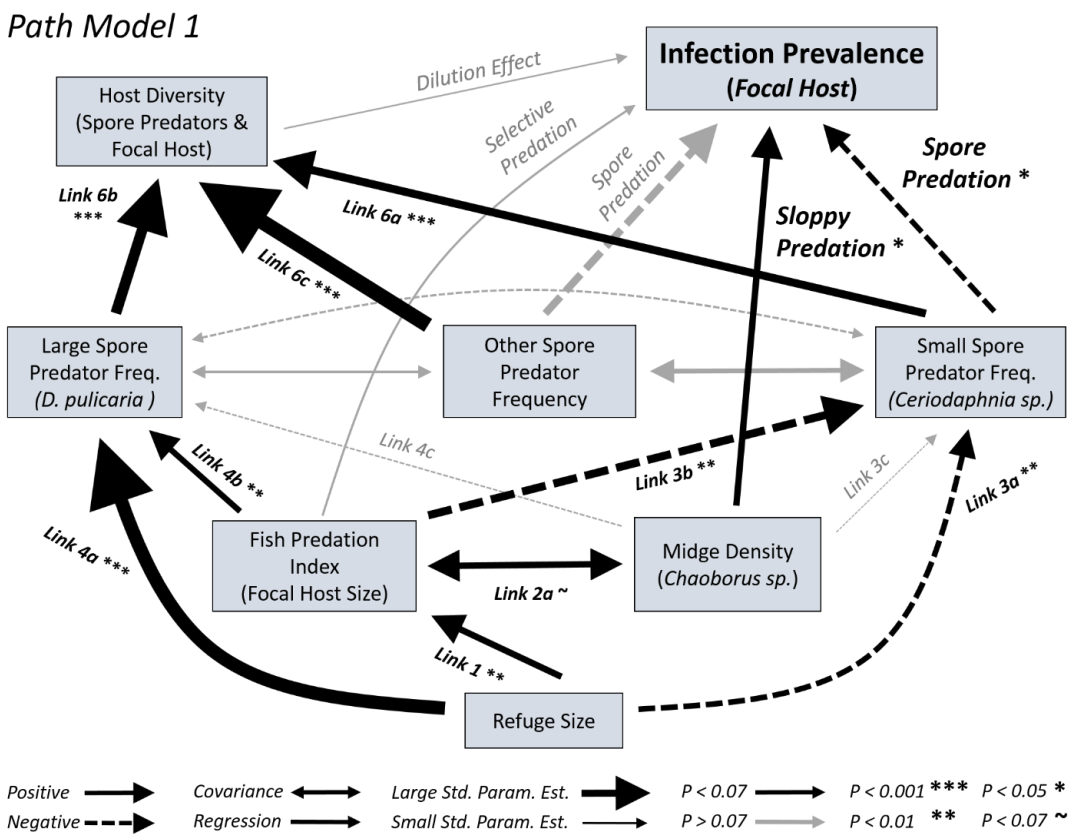

Figure 6. Path model 1 disentangles drivers of infection prevalence in a focal host (*Daphnia dentifera*). Ecological links among habitat, predators, and host diversity (Links 1-4 & 6, Table 2; Figs. 3 & 5) synthesize three modes of predation (Table 1; Fig. 2). From the bottom, moving up: **1)** Small refuges led to intense selective fish predation. **2a)** Intense fish predation correlated with low density of sloppy midge predators *(Chaoborus*). **3a)** Small refuges & **3b)** intense fish predation increased frequency of small spore predators (*Ceriodaphnia*) in the host community. **4a)** Large refuges & **4b)** less intense fish predation increased frequency of large spore predators (*D. pulicaria*). **6a-c)** Frequencies of all spore predators increased host diversity. **Disease Drivers:** Sloppy midge predators and small spore predators (*Ceriodaphnia*) had large, significant, and direct effects on infection prevalence. Selective fish predation did not directly drive infection prevalence, but indirectly mediated density of sloppy midge predators and frequency of small spore predators. Other spore predators reduced disease, but not significantly. The dilution effect pattern was not significant, once accounting for the direct effects of small spore predators and other spore predators. Model fit statistics: Satorra-Bentler chi square $P = 0.903$; CFI = 1.000; TLI = 1.152; RMSEA = 0.000; SRMR = 0.044.

this spurious pattern merely echoed, as a correlational shadow, direct links between infection prevalence and small spore predators (see Table 2).

Path models 2 and 3: Disease drivers and underlying ecological links

Model 2 (Fig. 7 A) disentangled drivers of density of infected hosts (Figs. 1 & S1). All analogous ecological links were identical (Links 1-2) or qualitatively similar (links 3-4) to model 1 (see Table 2). Additionally, (Link 5c) frequency of small spore predators (*Ceriodaphnia*) marginally correlated with lower density of focal hosts ($P = 0.070$; SPE = -0.240). In contrast, disease drivers differed extensively from Model 1. High total density of focal hosts caused high densities of infected focal hosts ($P < 0.001$; SPE = 0.500). Neither small spore predators ($P = 0.16$; SPE = -0.116), sloppy midge predators ($P = 0.19$; SPE = 0.190), nor selective fish predation ($P = 0.68$; SPE = 0.054) significantly regulated density of infected hosts, even though all appeared important univariately (Fig. S1 A-C). Instead, in this path model, the tight relationship between total and infected density of focal hosts (Fig. 1 B) washed out direct effects of those other drivers. Nevertheless, small spore predators indirectly reduced density of infections by marginally lowering density of infected hosts, most likely via competition. As in model 1, these small spore predators were regulated by habitat structure (refuge size) and fish predation (see Table 2). Thus, habitat structure still connected to disease through predator-mediated pathways. However, when predicting density of infected hosts, these connections became weaker and less direct.

Path model 3, the prevalence based analogue of model 2, largely mirrored the original model of infection prevalence (path model 1). For example, sloppy midge predators still directly influenced disease, and selective predators still exerted habitat-mediated indirect effects on infection prevalence through midges and small spore predators. However, the intentional contrasts between models 2 (Fig. 7 A) and 3 (Fig. 7

B) become uniquely informative. Both model structures linked small spore predators to focal host density and each respective disease metric. However, only the direct link to prevalence mattered in model 3 (since total density of focal hosts remained unconnected to infection prevalence). In contrast, only the indirect link mediated by density of focal hosts mattered in model 2 (since the link between densities of total and infected hosts was so strong). Thus, small spore predators reduced each disease metric through different pathways.

DISCUSSION

We disentangled drivers of zooplankton epidemics using a two-step approach, guided by theory and field data. In step one, we identified several potential disease drivers with univariate field patterns. In this analysis, host density was correlated with density of infected hosts, but not infection prevalence (Fig. 1). Additionally, both metrics correlated with selective fish predation, sloppy midge predation, and spore predation by certain zooplankton taxa (Fig. 2 & S1 A-E). Finally, both metrics declined with higher diversity of hosts (i.e., focal hosts and all spore predators combined). This univariate diversity-disease pattern supports a dilution effect (Fig. 2 & S1 F). However, some of these strong univariate patterns proved misleading, due to complex community interactions that obscured the direct and indirect drivers of disease (Figs. 3-5). In step two, path analysis uncovered and explained these misleading patterns. Specifically, path analyses delineated three types of complicating community interactions: 1) trophic interactions among predators (see Fig. 3), 2) impacts and regulators of focal host density (see Fig. 4), and 3) a spurious diversity-disease pattern (see Fig. 5). All of these interactions were ultimately grounded in habitat structure (i.e., refuge size; see Figs. 6-7).

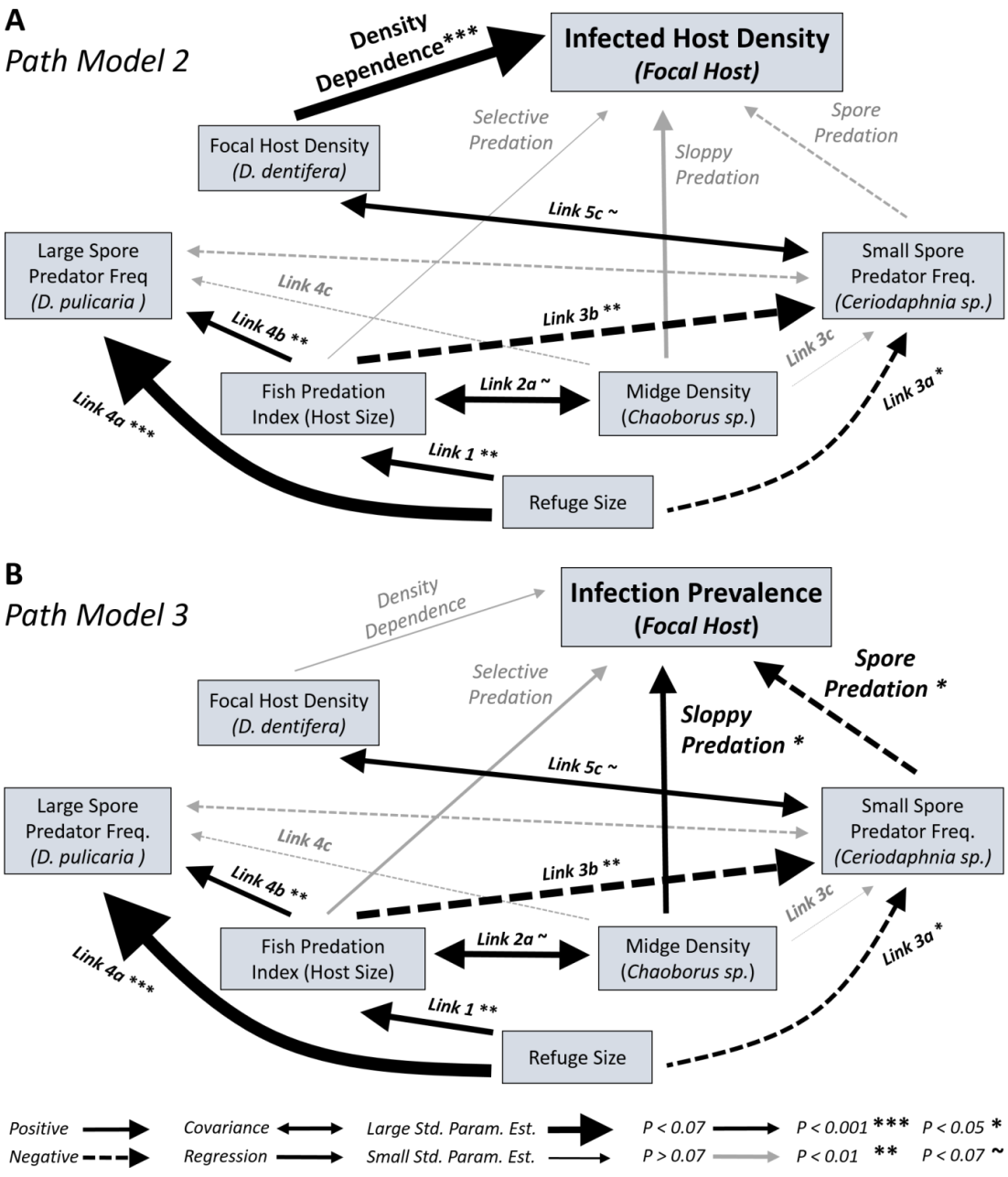

Figure 7. A) Path model 2 disentangles drivers of infected focal host density (*Daphnia dentifera*). **B)** Path model 3 mirrors the structure of model 1 (Fig. 6), but without 'host diversity', in order to facilitate direct comparisons with path model 2. **Both models:** Ecological links among habitat, host density, and predators (Links 1-5, Table 2; Figs. 1, 3 & 4) synthesize three modes of predation (Table 1; Fig. S1). Links 1-4 are qualitatively identical to Fig. 6. Additionally, **5c)** high frequencies small spore predators (*Ceriodaphnia* competitors) marginally correlated with low focal host densities. **Model**

2): Neither spore predators, sloppy predators, nor selective predators regulated density of infected hosts. Instead, it depended only on total density of focal hosts. **Model 3):** Drivers are qualitatively identical to model 1 (Fig. 6). Model 2 fit statistics: Satorra-Bentler chi square *P* = 0.317; CFI = 0.985; TLI = 0.948; RMSEA = 0.053; SRMR = 0.070. Model 3 fit statistics: Satorra-Bentler chi square *P* = 0.404; CFI = 0.997; TLI = 0.990; RMSEA = 0.022; SRMR = 0.066.

Path analysis improved our interpretation of univariate field patterns by breaking down each of these complicating community interactions. First, it clarified how trophic interactions among predators shaped disease. Surprisingly, in path models 1 and 3, selective fish predation did not directly reduce infection prevalence (despite Fig. 2 A). Instead, fish predation worked indirectly by decreasing density of sloppy midge predators (Link 2a; Fig. 3 B) and increasing frequency of small spore predators (Link 3b; Fig. 3 E). In turn, these indirect effects were modulated by size of the refuges from fish predators (Link 1; Fig. 2 A). Second, in path models 2 and 3, small spore predators drove the two disease metrics through fundamentally different pathways. Small spore predators directly reduced infection prevalence, but indirectly reduced density of infected hosts by lowering density of focal hosts (likely via competition, and marginally significant; Link 5c; Fig. 4 C). Finally, path model 1 undermined a causal interpretation of the dilution effect. Instead, the spurious univariate diversity-disease pattern merely reflected the direct effects of small spore predators on infection prevalence. In turn, these small spore predators were regulated by habitat structure and fish predation. Each of these results is more thoroughly discussed in turn.

Links 1-4): Trophic interactions among predators regulate direct and indirect effects on disease

Selective fish predation, regulated by habitat (Link 1; see Fig. 3 A), structured communities of other predators in these lakes as predicted (see Table 2). In lakes with small refuges, stronger fish predation reduced midge density (Link 2a; Fig. 3 B). Small bodied spore predators (*Ceriodaphnia*) became more frequent with smaller refuges and more intense fish predation (Links 3a&b; Fig. 3 D & E), while large spore predators (*D. pulicaria*) became more common with larger refuges and less intense fish predation (Links 4a&b; Fig. 3 G & H). Despite some suggestive univariate relationships (Links 3c & 4c; Fig. 3 F & I), midges had no effect on composition of spore predators in path models. Therefore, selective fish predators had the greatest capacity to regulate disease through trophically-mediated indirect interactions (i.e., predation on midges and spore predators). In other systems, other selective predators appear to regulate schistosomiasis (Sokolow et al. 2015), salmon lice (Krkosek et al. 2011), grasshopper fungus (Laws et al. 2009), moose tapeworms (Joly and Messier 2004), and grouse nematodes (Hudson et al. 1992) (see Table 1). In most of these systems, any potential indirect effects of these predators are less clear. However, their indirect effects could even be more important than their apparent direct effects, as in our case study here.

Indeed, indirect paths linking predators to disease apply broadly. First, our larger selective predator influenced density of the smaller sloppy predator. In turn, lakes with less fish predation had more disease via higher midge density (Figs. 6 & 7B). Related relationships among predators regulate other diseases. For example, foxes may reduce Lyme disease by lowering density of small mammal hosts that critically spread infection. However, coyotes can outcompete foxes, release small mammals from predation pressure by foxes, and indirectly elevate Lyme disease risk through these cascading

interactions (Levi et al. 2012). Similarly, lobster predators prevent epidemics in sea urchins by maintaining low densities of hosts. However, overharvesting lobsters releases urchins from predation pressure, stimulates their population growth, and indirectly promotes bacterial epidemics (Lafferty 2004). In all three cases, top predators (fish, coyotes, humans) mediate the impacts of mesopredators (midges, foxes, lobsters) on disease. Interestingly, mesopredators can then alter disease through different mechanisms, either increasing it (midges: by spreading parasites during sloppy feeding) or decreasing it (foxes and lobsters: by controlling density of key hosts).

Second, selective fish predators also regulated disease through direct shifts in the host community. Specifically, higher frequencies of small spore predators (*Ceriodaphnia*) reduced infection prevalence, likely via consumption of free-living parasites (Fig. 2 C). In turn, intense fish predation increased frequency of these small spore predators and hence indirectly reduced disease (Figs. 6 & 7B). Consumers in other systems can regulate disease via similar shifts in host communities. Grazing by vertebrate herbivores can increase frequency of highly competent grass hosts, and hence increase prevalence of viral disease (Borer et al. 2009). Thus, consumer mediated shifts in host communities can either increase or decrease disease. Other examples merit more thorough exploration. For example, variation in community structure of hosts can drive hantavirus transmission (Clay et al. 2009). Predators of rodents also appear to decrease hantavirus prevalence (Orrock et al. 2011). Could predators reduce hantavirus by regulating host community structure, by depressing density of focal hosts, or both?

Shifts in structure of host communities do not always drive disease. In our case study, large spore predators (*D. pulicaria*), had no effect on either disease metric (Figs. 2 & S1 D). This seemed surprising, since large spore predators completely resist infection and reduce transmission in experiments (Hall et al. 2009). In the field, they also reduced

epidemic size in a different set of Michigan lakes (Hall et al. 2009) and delayed the start of epidemics in a subset of the present Indiana lakes (Penczykowski et al. 2014). However, using seasonal averages, they did not reduce infection prevalence among lakes in Michigan (Hall et al. 2010) or Indiana (Fig. 2 D). Perhaps seasonal declines in refuge size in these Indiana lakes squeeze out this larger spore predator just as epidemics in the focal host begin. Alternatively, *D. pulicaria* can inhabit a deeper water microhabitat (Leibold 1991), potentially below where spores are consumed by focal hosts (Cáceres et al. 2009). Either way, large spore predators somehow remained temporally or spatially irrelevant. Nonetheless, a general lesson arises here: competency assays and transmission experiments alone may not identify key species that drive disease in nature. Experiments must be paired with field data to robustly identify these taxa (e.g., Johnson et al. 2013, Venesky et al. 2014, Rohr et al. 2015). Only then can we begin to sort through the direct and indirect species interactions that regulate disease.

Overall, indirect effects overshadowed the direct effects of selective fish predation in our case study. Initially, selective fish predation seemed to strongly regulate both metrics of disease (Fig. 2A, S1A). However, these univariate patterns (especially for infection prevalence) ignored trophic interactions between fish predation, midges, and small spore predators (described above). After accounting for these indirect effects in path model 1, the direct effects of fish predation disappeared (Figs. 6-7). Direct effects of fish predation might be more important elsewhere (e.g., in Michigan lakes: Duffy and Hall 2008, Hall et al. 2010). Alternatively, indirect effects mediated by mesoscale predators and host community structure might frequently overshadow direct effects of selective predators, even in the Michigan lakes (see Hall et al. 2010), or even more generally, in other disease systems (Table 1). Thus, our case study illustrates a

common challenge for community and disease ecologists. Focusing on potential direct effects of predators is relatively simple, while unraveling complicated trophic webs requires a great amount of data and insight from natural history. Nevertheless, these indirect effects can be extremely influential (e.g., Lafferty 2004, Borer et al. 2009, Levi et al. 2012, Orlofske et al. 2012, Orlofske et al. 2014, Rohr et al. 2015).

Link 5): Impacts and regulators of focal host density

Density of focal hosts impacted the two disease metrics differentially. Univariately, density of focal hosts had no relationship with infection prevalence (Fig. 1 A). However, total and infected density of focal hosts were closely linked (Fig. 1 B). This mismatch may have arisen because high host density can depress per capita infection risk, decoupling the density-prevalence relationship (Civitello et al. 2013). These different roles of host density caused stark differences between path models disentangling infection prevalence (path model 2; Fig. 7 A) and density of infected hosts (path model 3; Fig. 7 B). Specifically, small spore predators and sloppy midge predators directly regulated infection prevalence, but no predators directly regulated density of infected hosts. Instead, these potential impacts (supported univariately) were statistically overwhelmed by the strong link between density of total and infected hosts in the path analysis. In turn, focal host density was not regulated by fishes, midges, or large spore predators (Fig. 4 A, B & D, respectively). However, it was marginally regulated by frequency of small spore predators (Link 5c; Fig. 4 C; $P = 0.07$), who compete with focal hosts (Strauss et al. 2015) and who themselves depend on habitat structure and fish predation. Thus, these small spore predators indirectly reduced density of infected hosts, likely via competition (Fig. 7 A).

Consequently, small spore predators reduced disease in two different ways, each primarily driving a different disease metric. In general, consumption of free living fungal spores can *reduce encounters* between focal hosts and parasites, while competition can *regulate host density* (see Strauss et al. 2015). This combination of encounter reduction and host regulation defines 'friendly competition' (Hall et al. 2009, Strauss et al. 2015). Here, path analysis enabled us to partition host regulation (mediated by focal host density; Fig. 7B) versus encounter reduction (not mediated by focal host density; Fig. 7A). The partition reveals that host regulation primarily reduced density of infected hosts, while encounter reduction reduced infection prevalence. Thus, although the univariate links between *Ceriodaphnia* frequency and prevalence (Fig. 2 C) or density of infections (Fig. S1 C) looked superficially similar, they likely arose by different mechanisms. These two components of friendly competition may be quite general. Examples likely include hantavirus transmitted among rodents (Clay et al. 2009), *Schistosoma* among snails (Johnson et al. 2009), parasites in intertidal communities (Johnson and Thieltges 2010), emerging diseases in amphibians (Johnson et al. 2013, Venesky et al. 2014), and fungal pathogens and viruses in plant communities (Mitchell et al. 2002, Boudreau 2013, Lacroix et al. 2014). A similar partition between host regulation and encounter reduction could help clarify drivers of prevalence versus density of infections in all of these systems.

More generally, path analyses can attribute changes in disease to either changes in host density or changes in other drivers. This approach could be broadly useful (see Begon 2008). For example, it could determine whether selective predators (see Table 1) reduce disease by merely reducing total host density, or also by selectively culling infected hosts (or, as in this case study, via other indirect paths). In Lyme disease, density of infected ticks depends on both total tick density and infection prevalence. In

turn, both of these factors can depend on the rodent community (Vanbuskirk and Ostfeld 1995, Randolph and Dobson 2012). Path analysis could clarify whether rodents in field data drive Lyme disease more through infection prevalence or total density of ticks. Dragonfly predators regulate *Ribeiroia* infections in amphibians by both consuming free-living parasites (reducing transmission) and lowering host density via predation (elevating per-host transmission risk, because parasites seek hosts). These impacts counterbalance each other and are extremely difficult to detect in field data, but path models might tease them apart (Orlofske et al. 2014, Rohr et al. 2015). These examples exhibit a wide range of insights that can be gained with path models that distinguish between drivers of host densities and drivers of per capita transmission.

Link 6): Spurious diversity-disease pattern

The host diversity-disease pattern in our case study proved fairly misleading. In univariate regressions, higher diversity of hosts appeared to decrease prevalence (Fig. 2 F) and density (Fig. S1 F) of infections, consistent with the pattern behind the controversial dilution effect (Ostfeld and Keesing 2000a, Keesing et al. 2006, Begon 2008, Randolph and Dobson 2012). However, in path model 1 (Fig. 6), diversity had a negligible effect on disease. As such, our results support the dilution effect as spurious correlational pattern, but not a causal disease driver. Instead, path model 1 shows how small spore predators (*Ceriodaphnia*) strongly reduced infection prevalence themselves (Fig. 2 C & E). Simultaneously, frequency of all spore predators increased host diversity (Links 6a&c; Fig. 5 A & C). Once we accounted for these links, diversity itself had a negligible effect on disease. This result makes sense since no *a priori* mechanism links diversity *per se* to disease (see LoGiudice et al. 2003, Randolph and Dobson 2012). In contrast, *Ceriodaphnia* spore predators can reduce disease mechanistically—by both

consuming free-living parasite spores and competing with focal hosts (Strauss et al. 2015).

More generally, a similar confounding correlation between diversity and key 'diluters' can arise whenever focal hosts are common and diluters are rare (e.g., Ostfeld and Keesing 2000b, Johnson et al. 2013, Lacroix et al. 2014). Incidentally, this condition is one of the core requirements for a dilution effect (Ostfeld and Keesing 2000a, Keesing et al. 2006). Although meta-analysis demonstrates that diversity appears to broadly inhibit parasites (Civitello et al. 2015a), the mechanistic drivers of these diversity-disease patterns are rarely dissected. In the meta-analysis, 89 of 168 studies compared infection risk for host species with and without one additional species. In these cases, the design clarifies which 'diluter' species reduced disease. However, in the remaining 79 studies, it is often challenging to disentangle diversity *per se* from the identity of key diluters, especially in observational studies. Thus, compelling diversity-disease patterns of dilution effects may broadly obscure the key taxa and mechanisms driving these patterns. More experiments that independently manipulate diversity and species identity are needed to rigorously attribute 'diluting' effects to key taxa versus diversity *per se*.

Alternatively, with path analyses it even becomes possible to attribute *observational* dilution patterns to key diluter taxa. Through the same approach, we can also tease apart effects of key diluters from potential correlative changes in density of focal hosts (see Begon 2008). Finally, it becomes possible to link habitat to disease via key diluters (i.e., small predators dilute in higher predation lakes with smaller refuges). With this habitat-centered approach, we can clarify why species diversity correlates with disease, which species drive the pattern, and how they interfere with disease transmission. This approach greatly improves upon more correlative studies between diversity and disease (e.g., Allan et al. 2009, Huang et al. 2013), although those patterns offer an important starting point.

Future directions

The habitat-centered approach here could be expanded to synthesize other community interactions. For example, other habitat variables and abiotic drivers could explain additional variation in our *Metschnikowia* disease system. Here, we grounded all drivers in size of the deep water refuge. However, midge density was not related to refuge size (Link 2b; Fig. 3 C), possibly because midge larvae can also use deep anoxic waters or sediments below the deep-water refuge (Gonzalez and Tessier 1997). Instead, lakes with more dissolved organic carbon (DOC) have more midges (Overholt et al. 2012). DOC can also structure the refuge habitat, intensity of fish predation, and frequencies of spore predators in the cladoceran community (Wissel et al. 2003, Penczykowski et al. 2014). Moreover, DOC reduces solar radiation, which can directly kill free-living fungal *Metschnikowia* spores (Overholt et al. 2012). We aim to study these interactions in future analyses armed with more data. More ambitiously, we hope to eventually synthesize our results with other, less well-documented factors among our lakes. For example, a broader synthesis could incorporate impacts of human fishing, predation by piscivorous fish, lake productivity, shifts in phytoplankton communities, or outbreaks of other parasites of zooplankton, phytoplankton, or fishes. We must first lay the groundwork to understand all of these factors' roles in the aquatic food web before we can synthesize their interactions (but see Civitello et al. 2015b)

Path models of other disease systems could also test other important modes of predation. Most obviously, in other systems, predation of intermediate hosts could influence transmission of tropically-transmitted parasites while 'micropredation' can transmit parasites when micropredators act as disease vectors (see Lafferty and Kuris 2002). In our system, two additional modes may occur. First, predators can change host behavior, which may in turn change their exposure to parasites (Thiemann and

Wassersug 2000). Fish and midge predation can regulate the depths at which focal hosts and spore predators migrate and reside (Leibold 1991, Gonzalez and Tessier 1997), possibly influencing contact with parasites. Second, predators can change host traits, rendering them either more (e.g., Katz et al. 2014) or less (e.g., Groner and Relyea 2015) susceptible to parasites. One such trait is body size: larger hosts have higher exposure rates and larger spore yields, both of which can increase disease (Hall et al. 2007, Duffy et al. 2011, Bertram et al. 2013, Civitello et al. 2015b, Strauss et al. 2015). To understand how these and other modes of predation interact, we must first clearly understand their direct effects on disease (e.g., Table 1). Then, we can begin to examine their interactions.

Summary

Here, we disentangled community disease drivers of zooplankton epidemics using a two-step approach. We aimed to explain the most important paths linking habitat structure to disease, via changes in host density, three modes of predation, and/or host diversity. In step one, we identified several potential disease drivers with univariate field patterns, motivated by natural history theory. However, several of these univariate patterns proved misleading, due to complex community interactions. In step two, path analysis uncovered and explained these misleading patterns. For instance, we detected an apparent effect of selective predation, but then explained it better through indirect trophically-mediated effects on sloppy and spore predators. We detected weak effects of selective, sloppy, and spore predation on density of infected hosts, but these signals were overwhelmed by the much stronger signal of total host density itself. Finally, we detected a disease-diversity pattern signaling a ‘dilution effect’, but then explained the pattern mechanistically by encounter reduction and host regulation from a key spore predator taxa. Ultimately, habitat structure grounded all three of these

interactions in the path models. We hope that this approach to simplifying complexity will stimulate similar work in other disease systems. We must continue to disentangle these webs of interactions in order to advance our broad understanding of the community ecology of disease.

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CHAPTER 1 SUPPORTING INFORMATION

Appendix S1

In this Appendix, we first show correlations between three modes of predation and density of infected focal hosts (*Daphnia dentifera*). Then, we provide additional details of our path models. First we summarize the test statistic criteria used to judge each model (Table S1). Then, we report all parameters of the path model predicting infection prevalence including host diversity (path model 1; Fig. 6; Table S2), the model predicting density of infected hosts (path model 2; Fig. 7 A; Table S3), and its analogue predicting infection prevalence without host diversity (path model 3; Fig. 7 B; Table S4).

Table S1. Test statistics, cutoff criteria for determining good model fit, and statistics of all three path models (Figs. 6-7). Test statistics exceeding the desired cutoff criteria confirm that the hypothesized model is a relatively good fit for the observed data (Hu and Bentler 1999). All results use robust Satorra-Bentler chi square (Satorra and Bentler 2001).

Test Statistic	Desired Cutoff	Model 1 (Fig. 5)	Model 2 (Fig. 7A)	Model 3 (Fig. 7B)
Satorra-Bentler Chi Square	*P* value > 0.05	*P* = 0.903 [1]*df* = 9	*P* = 0.317 *df* = 6	*P* = 0.404 *df* = 6
Comparative Fit Index (CFI)	CFI > 0.95	1.000	0.985	0.997
Tucker Lewis Index (TLI)	TLI > 0.95	1.152	0.948	0.990
Root Mean Square Error of Approx. (RMSEA)	RMSEA < 0.06 [2]	0.000 (0.000-0.066)	0.053 (0.000-0.180)	0.022 (0.000-0.163)
Standardized Root Mean Square Residual (SRMR)	SRMR < 0.08	0.044	0.070	0.066

[1] Key to abbreviations: *df* = degrees of freedom. [2] 90% confidence interval

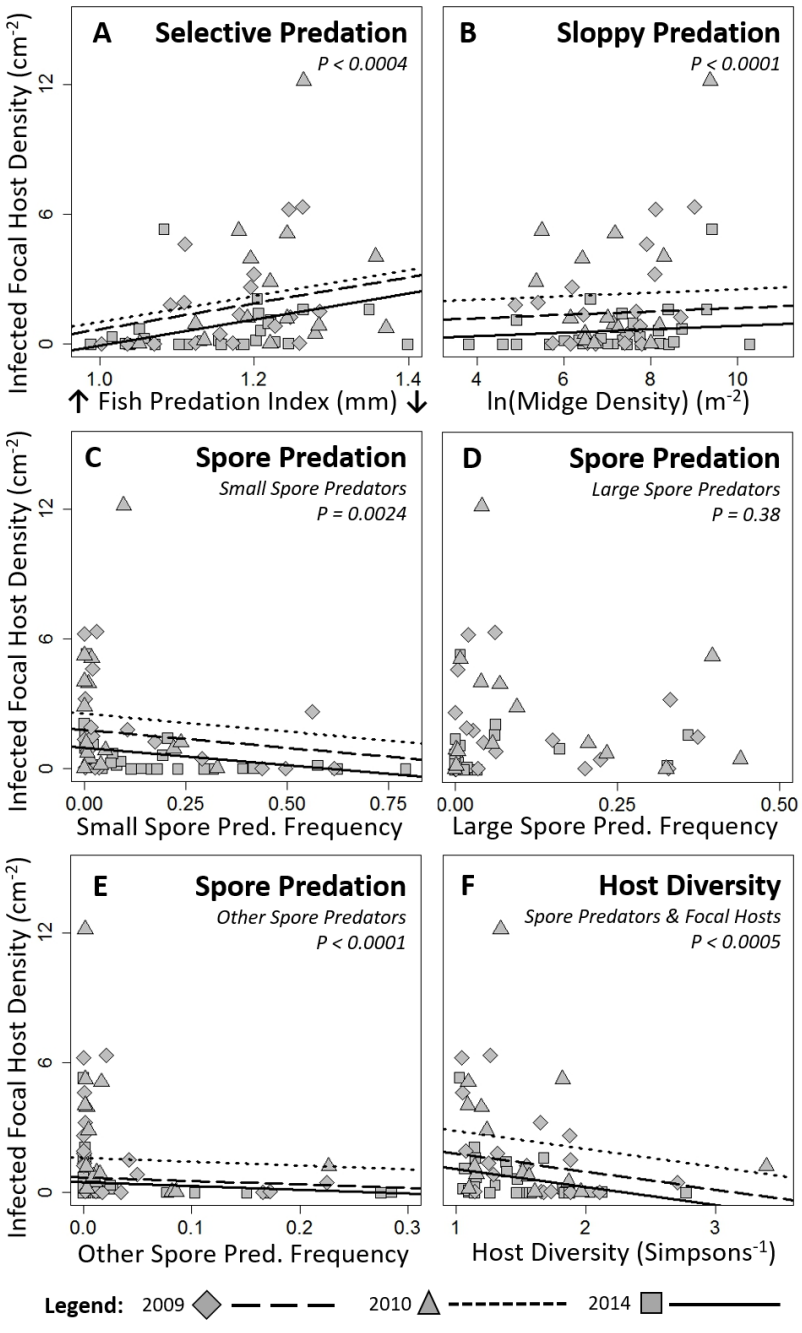

Figure S1. Three modes of predation (Table 1) are correlated with density of infected focal hosts (*Daphnia dentifera*). Infected host density is mean number of infected focal hosts per square centimeter during an epidemic season. Each point is a lake population in a given year. **A)** *Selective Predation:* Fish predation is indexed by body size of adult focal hosts (mm). Smaller size = more fish predation (↑); larger size = less (↓). More fish predation (left on x-axis) correlated with lower density of infected hosts. **B)** *Sloppy Predation:* More midge predators (*Chaoborus*) correlated with density of infected hosts. **C-E)** *Spore Predation:* Density of infected hosts dropped with **C)** higher frequencies small spore predators (*Ceriodaphnia*), but **D)** did not change with frequency of large spore predators (*D. pulicaria*). **E)** It also dropped with higher frequencies of other spore predators. *Host Diversity:* **F)** Higher cladoceran diversity also correlated with lower density of infected focal hosts, consistent with a dilution effect. Regression models were fit with random 'lake' effects, fixed 'year' effects, and flexible variance functions to account for heteroscedasticity in the data.

Table S2. Parameters for the path model predicting infection prevalence in focal hosts, including host diversity as a driver (path model 1; Fig. 6). Bold lines indicate significant or trending relationships.

Dep. Var.[1] / Model Component	Explanatory Variable	Par.[1] Est.	SE[1]	Z-value (Wald Statistic)	*P*[1]	Stand. Par. Est.[1]
Infection Prevalence ~	**Small Spore Predators**	**-0.094**	**0.047**	**-1.981**	**0.048**	**-0.231**
	Fish Predation Index	0.082	0.113	0.720	0.471	0.098
	Midge Density	**0.017**	**0.008**	**2.231**	**0.026**	**0.294**
	Other Spore Predators	-0.359	0.220	-1.633	0.103	-0.332
	Host Diversity	0.011	0.040	0.271	0.786	0.063
Host Diversity ~	**Other Spore Predators**	**4.212**	**0.873**	**4.824**	**0.000**	**0.664**
	Small Spore Predators	**0.868**	**0.269**	**3.230**	**0.001**	**0.365**
	Large Spore Predators	**1.754**	**0.149**	**11.787**	**0.000**	**0.479**
Small Spore Predators~	**Fish Predation Index**	**-0.718**	**0.232**	**-3.099**	**0.002**	**-0.351**
	Refuge Size	**-0.038**	**0.015**	**-2.621**	**0.009**	**-0.251**
	Midge Density	-0.006	0.018	-0.322	0.747	-0.039
Large Spore Predators~	**Fish Predation Index**	**0.337**	**0.109**	**3.093**	**0.002**	**0.254**
	Refuge Size	**0.059**	**0.013**	**4.405**	**0.000**	**0.600**
	Midge Density	-0.007	0.007	-1.038	0.299	-0.075
Fish Predation Index~	**Refuge Size**	**0.022**	**0.008**	**2.853**	**0.004**	**0.297**
Modeled	Sp. Pred. 1 ~~ Sp. Pred. 2	-0.001	0.001	-0.986	0.324	-0.085

Covariances:	Sp. Pred. 2 ~~					
	Other Sp. Pred.	0.001	0.001	1.211	0.226	0.108
	Sp. Pred. 1 ~~					
	Other Sp. Pred.	0.003	0.002	1.334	0.182	0.216
	Fish Pred. ~~					
	Midge Density	**0.033**	**0.017**	**1.942**	**0.052**	**0.281**
Intercepts:	Infection					
	Prevalence	-0.140	0.128	-1.089	0.276	-1.801
	Host Diversity	1.077	0.063	16.985	0.000	2.369
	Small Spore					
	Predators	1.087	0.241	4.511	0.000	5.680
	Large Spore					
	Predators	-0.342	0.111	-3.078	0.002	-2.757
	Fish Predation					
	Index	1.146	0.018	62.048	0.000	12.240
	Midge Density	7.162	0.233	30.677	0.000	5.474
	Other Spore					
	Predators	0.040	0.014	2.852	0.004	0.564
	Refuge Size	1.317	0.210	6.266	0.000	1.050
Variances:	Infection					
	Prevalence	0.004	0.001			0.696
	Host Diversity	0.038	0.013			0.185
	Small Spore					
	Predators	0.028	0.007			0.752
	Large Spore					
	Predators	0.008	0.002			0.490
	Fish Predation					
	Index	0.008	0.002			0.912
	Midge Density	1.711	0.350			1.000
	Other Spore					
	Predators	0.005	0.002			1.000
	Refuge Size	1.575	0.364			1.000

[1] Key to abbreviations: Dep. Var. = dependent variable; Par. Est. = parameter estimate; SE: = Standard error; *P* = *P*-value of parameter estimate; Stand. = standardized

Table S3. Parameters for the path model predicting density of infected focal hosts (path model 2; Fig. 7 A). Bold lines indicate significant or trending relationships.

Dep. Var.[1] / Model Component	Explanatory Variable	Par.[1] Est.	SE[1]	Z-value (Wald Statistic)	*P*[1]	Stand. Par. Est.[1]
Density of Infected Focal Hosts~	Small Spore Predators	-0.131	0.093	-1.414	0.157	-0.116
	Fish Predation Index	0.124	0.295	0.419	0.675	0.054
	Midge Density	0.031	0.024	1.319	0.187	0.190
	Focal Host Density	**0.854**	**0.267**	**3.199**	**0.001**	**0.500**
Small Spore Predators~	**Fish Predation Index**	**-0.723**	**0.276**	**-2.617**	**0.009**	**-0.358**
	Refuge Size	**-0.032**	**0.015**	**-2.091**	**0.037**	**-0.211**
	Midge Density	-0.002	0.017	-0.136	0.892	-0.016
Large Spore Predators~	**Fish Predation Index**	**0.312**	**0.105**	**2.965**	**0.003**	**0.236**
	Refuge Size	**0.060**	**0.013**	**4.477**	**0.000**	**0.608**
	Midge Density	-0.007	0.007	-0.934	0.350	-0.070
Fish Pred. Index~	**Refuge Size**	**0.022**	**0.008**	**2.853**	**0.004**	**0.297**
Modeled Covariances:	Sp. Pred. 1 ~~ Sp. Pred. 2	-0.002	0.001	-1.316	0.188	-0.120
	Sp. Pred. 1 ~~ Focal Host Dens.	**-0.005**	**0.003**	**-1.810**	**0.070**	**-0.240**
	Fish Pred. In. ~~ Midge Dens.	**0.033**	**0.017**	**1.942**	**0.052**	**0.281**
	Sp. Pred. 2 ~~ Focal Host Dens.	0.000	0.002	-0.255	0.799	-0.032
Intercepts:	Density of Infected Focal Hosts	-0.358	0.372	-0.962	0.336	-1.672

	Small Spore Predators	1.060	0.286	3.710	0.000	5.606
	Large Spore Predators	-0.317	0.108	-2.948	0.003	-2.567
	Fish Predation Index	1.146	0.018	62.048	0.000	12.240
	Midge Density	7.162	0.233	30.677	0.000	5.474
	Focal Host Density	0.182	0.017	10.836	0.000	1.456
	Refuge Size	1.317	0.210	6.266	0.000	1.050
Variances:	Density of Infected Focal Hosts	0.030	0.011			0.658
	Small Spore Predators	0.028	0.007			0.779
	Large Spore Predators	0.008	0.002			0.493
	Fish Predation Index	0.008	0.002			0.912
	Midge Density	1.711	0.350			1.000
	Focal Host Density	0.016	0.003			1.000
	Refuge Size	1.575	0.364			1.000

[1] Key to abbreviations: Dep. Var. = dependent variable; Par. Est. = parameter estimate; SE: = Standard error; *P* = *P*-value of parameter estimate; Stand. = standardized

Table S4. Parameters for the path model predicting infection prevalence without host diversity as a driver (path model 3; Fig. 7 B). Bold lines indicate significant or trending relationships.

Dep. Var. [1] / Model Component	Explanatory Variable	Par.[1] Est.	SE[1]	Z-value (Wald Statistic)	*P*[1]	Stand. Par. Est.[1]
Infection Prevalence ~	**Small Spore Predators**	**-0.104**	**0.053**	**-1.975**	**0.048**	**-0.253**
	Fish Predation Index	0.115	0.135	0.853	0.394	0.139
	Midge Density	**0.016**	**0.008**	**1.999**	**0.046**	**0.268**
	Focal Host Density	0.039	0.054	0.710	0.478	0.062

Small Spore	**Fish Predation Index**	**-0.723**	**0.276**	**-2.617**	**0.009**	**-0.358**
Predators~	**Refuge Size**	**-0.032**	**0.015**	**-2.091**	**0.037**	**-0.211**
	Midge Density	-0.002	0.017	-0.136	0.892	-0.016
Large Spore	**Fish Predation Index**	**0.311**	**0.105**	**2.951**	**0.003**	**0.235**
Predators~	**Refuge Size**	**0.060**	**0.013**	**4.548**	**0.000**	**0.609**
	Midge Density	-0.007	0.007	-0.933	0.351	-0.070
Fish Pred.	**Refuge Size**	**0.022**	**0.008**	**2.853**	**0.004**	**0.297**
Index~						
Modeled	Sp. Pred. 1 ~~ Sp.					
Covariances:	Pred. 2	-0.002	0.001	-1.309	0.191	-0.119
	Sp. Pred. 1 ~~ Focal					
	Host Dens.	**-0.005**	**0.003**	**-1.809**	**0.070**	**-0.240**
	Fish Pred. In. ~~					
	Midge Dens.	**0.033**	**0.017**	**1.942**	**0.052**	**0.281**
	Sp. Pred. 2 ~~ Focal					
	Host Dens.	0.000	0.001	0.105	0.916	0.019
Intercepts:	Infection Prevalence	-0.172	0.159	-1.084	0.278	-2.217
	Small Spore Predators	1.060	0.286	3.710	0.000	5.606
	Large Spore Predators	-0.317	0.108	-2.934	0.003	-2.560
	Fish Predation Index	1.146	0.018	62.048	0.000	12.240
	Midge Density	7.162	0.233	30.677	0.000	5.474
	Focal Host Density	0.182	0.017	10.836	0.000	1.456
	Refuge Size	1.317	0.210	6.266	0.000	1.050
Variances:	Infection Prevalence	0.005	0.001			0.769
	Small Spore Predators	0.028	0.007			0.779
	Large Spore Predators	0.008	0.002			0.492
	Fish Predation Index	0.008	0.002			0.912
	Midge Density	1.711	0.350			1.000
	Focal Host Density	0.016	0.003			1.000
	Refuge Size	1.575	0.364			1.000

[1] Key to abbreviations: Dep. Var. = dependent variable; Par. Est. = parameter estimate; SE: = Standard error; *P* = *P*-value of parameter estimate; Stand. = standardized

Chapter 2

Success, failure, and ambiguity of the dilution effect among competitors

Citation:

Strauss, A.T., D.J. Civitello, C.E. Cáceres, and S.R. Hall. 2015. Success, failure, and ambiguity of the dilution effect among competitors. *Ecology Letters*. 18: 916-926.

CHAPTER 2 ABSTRACT

It remains challenging to predict variation in the magnitude of disease outbreaks. The dilution effect seeks to explain this variation by linking multiple host species to disease transmission. It predicts that disease risk increases for a focal host when host species diversity declines. However, when an increase in species diversity does not reduce disease, we are often unable to diagnose why. Here, we increase mechanistic and predictive clarity of the dilution effect with a general trait-based model of disease transmission in multi-host communities. Then, we parameterize and empirically test our model with a multi-generational case study of planktonic disease. The model-experiment combination shows that hosts that vary in competitive ability (R^*) and potential to spread disease (R_0) can produce three qualitatively disparate outcomes of dilution on disease prevalence: the dilution effect can succeed, fail, or be ambiguous/irrelevant.

INTRODUCTION

Disease outbreaks can regulate dynamics of host populations (Anderson & May 1979) and shift the outcome of competition between species (Freeland 1983; Price *et al.* 1988). However, we still struggle to uncover how interactions among host species regulate disease (Holt *et al.* 2003). The dilution effect offers potentially powerful connections between host communities and transmission. In the broadest sense (Keesing *et al.* 2006), it predicts that a decline in diversity (fewer diluter species) elevates disease risk for a more vulnerable focal host. Diluter species can decrease transmission when infected vectors waste bites on diluters, when diluters remove environmentally distributed parasites (e.g., by eating propagules), when diluters depress focal host density (e.g., by depleting shared resources), or when diluters modify host behavior (Keesing *et al.* 2006; Keesing *et al.* 2010). All of these proposed 'local dilution mechanisms' reduce contact between focal hosts and parasites. Hence, losses of diluter species can elevate host-parasite contact, transmission, and the severity of disease outbreaks.

Evidence for dilution has now arisen in numerous systems. Some involve risks to human health, including Lyme disease (Ostfeld & Keesing 2000; LoGiudice *et al.* 2003), West Nile virus (Allan *et al.* 2009), Schistosomiasis (Johnson *et al.* 2009), and Hanta virus (Clay *et al.* 2009; Suzan *et al.* 2009). Other diseases strictly infect plant and wildlife hosts (Mitchell *et al.* 2002; Johnson *et al.* 2008; Hall *et al.* 2009a; Johnson & Thieltges 2010; Johnson *et al.* 2013; Becker *et al.* 2014; Lacroix *et al.* 2014; Rottstock *et al.* 2014; Venesky *et al.* 2014). These examples indicate that further species losses may enhance disease risk in a variety of ecosystems. However, the dilution effect remains controversial, because higher species diversity does not always reduce disease. Sometimes diversity even amplifies disease (Keesing *et al.* 2006; Ogden & Tsao 2009; Wood *et al.* 2014). Additionally, switches between definitions of 'disease risk' (infection

prevalence versus density of infected hosts) can qualitatively change observation of a dilution effect (Begon 2008; Roche *et al.* 2012). Critiques of the dilution effect question its generality, robustness to the definition of 'disease risk', and spatial scale (Randolph & Dobson 2012; Salkeld *et al.* 2013; Wood & Lafferty 2013; Wood *et al.* 2014). More to the point, we still cannot predict when diversity *will* reduce disease. This problem arises especially when reports of the dilution phenomenon do not mechanistically pinpoint the underlying interactions that reduce disease (e.g. Allan *et al.* 2009; Clay *et al.* 2009). Thus, developing and testing a predictive, mechanistic framework for dilution could help us focus on *why*, rather than just *how frequently* dilution occurs.

Here, we take a modular approach to this problem, focusing on the traits and interactions among a few species. We develop and test a model of the interactions between two host species, their shared parasite, and resource. Thus, we narrow our focus to the local scale (sensu Holt *et al.* 2003), rather than a regional one (e.g., Johnson *et al.* 2013; Mihaljevic *et al.* 2014). Extant dilution models often assume asymmetries in species' epidemiological traits/parameters (e.g., Schmidt & Ostfeld 2001; Rudolf & Antonovics 2005; Ogden & Tsao 2009; Roche *et al.* 2012), and the most convincing empirical studies measure these traits (LoGiudice *et al.* 2003; Johnson *et al.* 2013; Lacroix *et al.* 2014). However, unlike most extant models (e.g., Dobson 2004; Keesing *et al.* 2006; Johnson & Thieltges 2010; Roche *et al.* 2012) and experiments (e.g., Johnson *et al.* 2008; Becker *et al.* 2014; Venesky *et al.* 2014; Wojdak *et al.* 2014), we allow our host species to dynamically interact and mechanistically influence each other's densities via these traits. Then, we explore a range of realistic outcomes in our community module (three case studies) by measuring intraspecific variation in the traits of our focal host (sensu Bolnick *et al.* 2011). Finally, we parameterize and test our model with corresponding multi-generational experiments. This novel, synthetic approach, highlights key interactions overlooked by other theory and experiments. We

show how community ecology (resource competition and R^*) and epidemiology (potential of disease spread, R_0) can govern the success, reveal a recurrent cost (competition), and unveil a potential byproduct (spillover) of local dilution. As a result, we push beyond the controversy toward a more mechanistic, experimentally-tested evaluation of the dilution effect.

To build this model, we return to those 'local dilution mechanisms' (Keesing *et al.* 2006; Johnson & Thieltges 2010; Keesing *et al.* 2010), and most importantly, their interactions. First, diluter species can *reduce encounters* between focal hosts and parasites. For parasites transmitted environmentally, this occurs via a 'vacuum mechanism': resistant diluter species remove parasites from the environment while rarely (or never) becoming sick. Through this removal, diluters lower the risk of infection for the focal host (Johnson & Thieltges 2010). Second, diluters can *regulate* focal host populations via competition for space or resources. All else equal, such regulation reduces density-dependent transmission for environmentally distributed parasites (Anderson & May 1981). These two mechanisms (encounter reduction and host regulation) operate simultaneously in the 'friendly competition module' (Hall *et al.* 2009a). Competition typically depresses fitness of both hosts; yet, in 'friendly competition' one competitor can indirectly benefit from reduced disease (i.e., parasite-mediated apparent facilitation). The friendly competition module must be widespread, since species often encounter the same parasites when competing for resources or space (Freeland 1983; Price *et al.* 1988). Examples likely include Hanta transmitted among rodents (Clay *et al.* 2009; Suzan *et al.* 2009), Schistosoma among snails (Johnson *et al.* 2009), parasites in intertidal communities (Johnson & Thieltges 2010), emerging diseases in amphibians (Johnson *et al.* 2013; Becker *et al.* 2014), fungal pathogens and viruses in plant communities (Mitchell *et al.* 2002; Lacroix *et al.* 2014; Rottstock *et al.* 2014), potentially important agricultural examples (Boudreau 2013), and,

at least theoretically, perhaps even Lyme disease (Ogden & Tsao 2009). Thus, a mechanistic understanding of dilution in many systems may require embracing 'friendly competition'.

At first glance, friendly competition seems destined to promote successful dilution. After all, friendly competition rests on two mechanisms – encounter reduction and host regulation – that both decrease transmission. Yet, interactions between these mechanisms pose four crucial uncertainties. First, focal hosts that compete strongly could constrain the density of competitor/diluters. Sparse competitor/diluters may not sufficiently reduce encounters of hosts with parasite propagules, particularly when focal hosts create large epidemics. Second, competitor/diluters (if not completely resistant) could then be overwhelmed with parasite propagules and suffer spillover (amplified disease) from uncontrolled focal host epidemics. Third, competition from diluters could strongly depress focal host density. Even in cases where competitor/diluters reduce infection prevalence, they could still decrease density of healthy (uninfected) focal hosts. Fourth, the relative cost of competition and benefit of dilution could vary by perspective, depending on the metric used to define 'disease risk' (infection prevalence versus density of infected hosts). Each uncertainty hinges on traits of species involved: how strongly focal hosts compete with diluters (R^*) and how effectively they spread disease (R_0).

Here, we compare three empirically-motivated case studies to explore the above uncertainties inherent in friendly competition. By allowing feedbacks among interacting species, we reveal that the outcome of dilution (measured both in terms of infection prevalence and density of infected hosts) does not simply mirror the additive effects of host regulation (competition) and encounter reduction (parasite removal). More specifically, we show that the outcome of dilution (success, failure, or ambiguity/irrelevance) depends on the interactions between a focal host's ability to

compete and its ability to spread disease. This pairing of theory and experiments offers novel insights into the friendly competition module, and brings predictive clarity to the dilution effect among competitors.

MATERIALS AND METHODS

Study system & Model specification

Our focal host zooplankter (*Daphnia dentifera*) non-selectively grazes on phytoplankton, and is the dominant grazer in many North American freshwater lakes (Tessier & Woodruff 2002). Across many of these lakes, this host experiences yearly epidemics of a virulent fungus *Metschnikowia bicuspidata* in late summer and fall (Hall *et al.* 2010b). *M. bicuspidata* can infect several zooplankton species, but we have only observed severe epidemics in our focal host species (Hall *et al.* 2009a). Community assembly of zooplankton in these lakes is predominantly determined by physical constraints (lake depth) and the degree of fish predation (Tessier & Woodruff 2002). Another zooplankter grazer, *Ceriodaphnia sp.*, co-occurs with our focal host in shallow lakes with some deep water refuge from fish predators (Tessier & Woodruff 2002). In lakes where our focal host and this competitor/diluter co-occur, epidemics tend to be smaller for the focal host. This observation offers tentative support for a dilution effect among these species (Hall *et al.* 2010b).

Friendly competition emerges inherently from this natural history, which we depict graphically (Fig. 1, center) and describe mathematically (Box 1). For a robust mathematical analysis of a similar model, see Cáceres *et al.* (2014). Susceptible focal hosts (S_{FH}) filter water at a foraging rate (f) and convert their algal resource (R) into births with conversion efficiency (e). While foraging non-selectively on algae, hosts inadvertently consume spores (Z) and thus become exposed to the virulent fungus *M.*

bicuspidata, also at rate (*f*) (Hall *et al.* 2007). Post exposure, susceptible focal hosts enter the infected class (*I*), with per-spore susceptibility (*u*). Once infected, these hosts cannot recover, and host death rate increases from parasite virulence (*v*). After death, hosts release a number (σ) of fungal spores back into the environment, fulfilling obligate killer epidemiology, common to a variety of disease systems (Ebert & Weisser 1997). Spore yield increases with resources (see Appendix S1 in Supporting Information for modeling details; Hall *et al.* 2009b). Critically, focal host genotypes vary in these traits, translating into variation in both competitive ability (R^*) and the potential for disease spread (R_0). Susceptible competitor/diluters *Ceriodaphnia sp.* ($S_{C/D}$) compete with focal hosts for algae, but strongly resist infection from consumed spores (Hall *et al.* 2010b). Thus, this competitor/diluter could reduce disease via spore vacuuming (encounter reduction) and/or competition (regulation of susceptible hosts). Competition also constrains the density of competitor/diluters, which limits their net vacuuming rate.

Trait measurements

In our mechanistic framework for friendly competition, traits ultimately determine the fate of the dilution effect. We measured critical traits (foraging/exposure rate *f*, conversion efficiency *e*, susceptibility *u*, virulence *v*, and spore yield σ) for three focal host genotypes and one diluter genotype of a separate species. All genotypes were chosen from existing laboratory cultures that had been isolated from lakes in southwestern Michigan. Using limited prior knowledge of these genotypes (Hall *et al.* 2010a), we selected our three focal host genotypes for our case studies that spanned a gradient of overall resistance to infection (exposure times susceptibility; $f \times u$). This provided us with the trait space necessary to explore a range of dilution outcomes.

Prior to trait measurement assays, all genotypes were grown in isoclonal cultures and fed high quality laboratory-cultured algae (*Ankistrodesmus falcatus.*). Cultures were maintained in filtered (Pall A/E: 1.0 µm) lake water under ideal conditions for three

generations in order to standardize any maternal affects. We estimated foraging rate (*f*) with a foraging assay; per-spore susceptibility (*u*) with an infection assay (Hall *et al.* 2010a); and conversion efficiency (*e*), virulence (*v*) and spore yield (σ) with a life table experiment (see Appendix S1 for details and parameter estimation; Fig. 1 A-D). We replicated trait measurement assays by genotype and bootstrapped 95% confidence intervals in R (R Development Core Team 2008).

Next, we summarized the traits of our focal host genotypes using model-derived indices of the potential for disease spread (R_0: Anderson & May 1981) and competitive ability (R^*: Tilman 1977). Strong competitors have low R^*'s (minimal resource requirements); strong disease spreaders have high R_0's (basic reproductive rates of the parasite). When combined, these two indices delineated three distinct phenotypes of the focal host (R_0: Fig. 1 E; R^*: Fig. 1 F). We featured these three phenotypes in each of the three case studies discussed below. Case 1 uses a focal host with low R^* and high R_0 (Fig. 1, first [light green] bars); case 2 uses a focal host with high R^* and moderate R_0 (Fig. 1, second [dark green] bars); case 3 uses a focal host with high R^* and low R_0; Fig. 1, third [purple] bars). The diluter had the lowest R^* and lowest R_0, indicating that it competed strongly but spread disease poorly (without complete resistance; Fig. 1 E,F, fourth [blue] bars).

Model predictions

Using our dynamical model (Box 1), we assessed whether the addition of the competitor/diluter reduced disease for each focal host, both in terms of infection prevalence and density of infected focal hosts. We simulated our model using the deSolve package in R. Parameters are defined in Box 1. Estimates for conversion efficiency *e*, foraging/exposure rate *f*, susceptibility *u*, virulence *v*, and spore yield σ varied among genotypes and were estimated with the assays described above (Fig. 1

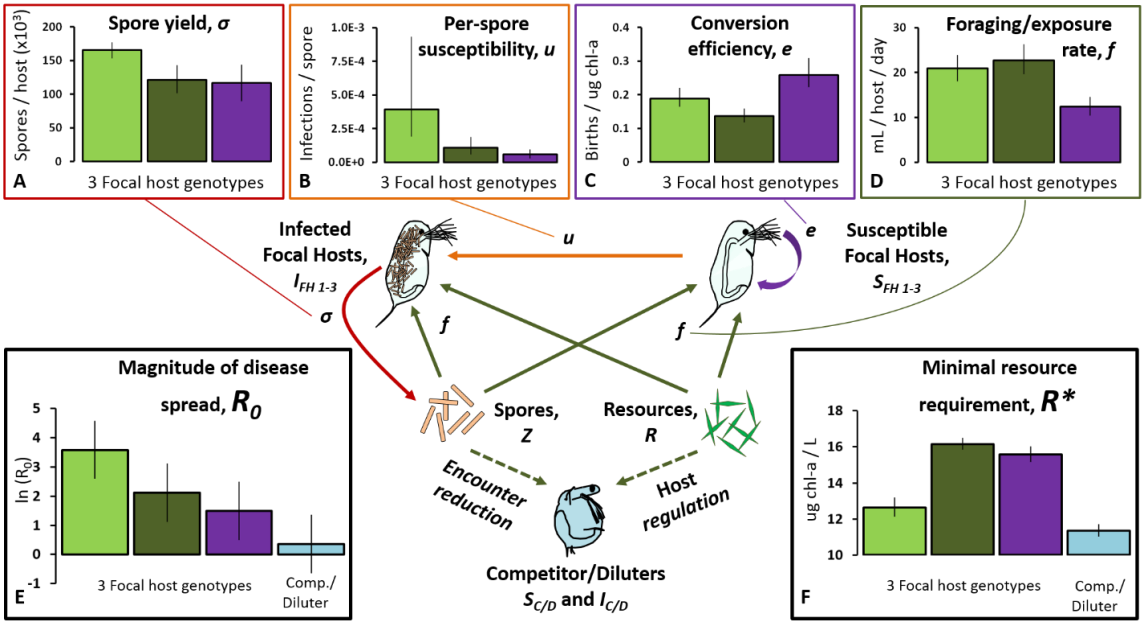

Figure 1. Focal host genotypes (indexed by FH 1-3) vary in four key traits which determine an index of disease spread (R_0) and an index of competitive ability (R^*). Infected focal hosts A) produce spore yield σ and B) become infected with per-spore susceptibilities u. Susceptible focal hosts C) convert resources into births with conversion efficiencies e and D) encounter algal resources and spores at foraging/exposure rates f. Competitor/diluters (indexed by C/D) reduce disease by consuming resources (host regulation) or spores (encounter reduction). Competitor/diluter traits are not shown. Variation in traits drives differences in focal host and competitor/diluter phenotypes, summarized as E) the potential for disease spread, R_0, and F) minimal resource requirements, R^*. (Strong competitors have low R^*s). Error bars are bootstrapped 95% confidence intervals. Differences among these focal host genotypes lead to the qualitative differences seen in the model simulations (left columns in Figs. 2-4).

and S1). Other parameter estimates (maximum algal growth rate r, algal carrying capacity K, spore loss rate m, and background death rate d) are described in Appendix S1. All simulations began with low density of focal hosts and/or diluters (S_{FH} = 1 L^{-1}, $S_{C/D}$ = 0 or 1 L^{-1}, R = 35 ug chl-a L^{-1}, and Z = 0 L^{-1}) and allowed hosts to increase in density for 15 days (as in the experiment below). Differences in densities on day 16 arose from

Box 1. A dynamical model describing changes in host, parasite and resource densities.

Susceptible hosts (S) are non-selective feeders and encounter parasites (Z) while foraging for resources (R). Parasites are obligate killers and hosts do not recover. Infected hosts (I) also forage and reproduce. Spore yield (σ) is a function of resources (Fig. S2). i = Focal Host 1-3 or the Competitor/Diluter. Traits (parameters for e_i, f_i, u_i, σ_i, and v_i) were measured with laboratory assays (Fig. 1). These differences cause qualitative differences in simulations (Figs. 2-4). For all simulations, background death rate d = 0.05; spore death rate m = 0.2; resource growth rate r = 0.9; resource carrying capacity K = 250.

Host dynamics:

Births: $e_i f_i R (S_i + I_i)$ — Deaths: $-d S_i$ — Transmission: $-u_i f_i Z S_i$

$$\frac{dS_i}{dt} = e_i f_i R (S_i + I_i) - d S_i - u_i f_i Z S_i$$

Transmission: $u_i f_i Z S_i$ — Increased mortality: $-(d + v_i) I_i$

$$\frac{dI_i}{dt} = u_i f_i Z S_i - (d + v_i) I_i$$

Parasite dynamics:

Parasite release: $\sum_i \sigma_i (R)(d + v_i) I_i$ — Parasite removal: $-\sum_i f_i Z (S_i + I_i)$ — Parasite loss: $-mZ$

$$\frac{dZ}{dt} = \sum_i \sigma_i (R)(d + v_i) I_i - \sum_i f_i Z (S_i + I_i) - mZ$$

Resource dynamics:

Res. growth: $rR\left(1 - \frac{R}{K}\right)$ — Resource removal: $-\sum_i f_i R (S_i + I_i)$

$$\frac{dR}{dt} = rR\left(1 - \frac{R}{K}\right) - \sum_i f_i R (S_i + I_i)$$

Definitions and units for parameters and variables:

S (susceptible host density; L^{-1})
I (infected host density; L^{-1})
Z (spore density; L^{-1})
R (resource dens.; µg chl-*a* L^{-1})
e (conv. efficiency; µg chl-*a*$^{-1}$)
f (foraging rate; L day^{-1})
d (death rate; day^{-1})
u (suscep.; hosts $spore^{-1}$)
v (virulence; day^{-1})
σ (spore yield; $host^{-1}$)
m (spore loss; day^{-1})
r (resource growth; day^{-1})
K (resource carrying capacity; µg chl-*a* L^{-1})

differences in traits between genotypes. On day 16, we simulated epidemics by adding spores (Z = 5,000 L^{-1}). We plotted infection prevalence and log transformed infected host density and uninfected host density over the first 31-35 days of the epidemics, according to the length of each corresponding mesocosm experiment.

Mesocosm experiments

Parallel experiments grew isoclonal populations of each focal host genotype, both alone and with the competitor/diluter. Mesocosm experiments were housed in 75-liter acid-washed polyethylene tanks in a climate-controlled room and grown under a 16 L: 8 D light cycle. Tanks were filled to 60 liters with a mixture of 80% tap water (detoxified with Kordon Amquel Plus and Novaqua Plus) and 20% filtered lake water. Evaporated water was replaced throughout the experiments. Initial doses of nitrogen and phosphorus were added to the tanks in the form of sodium nitrate and potassium phosphate (300 ug L^{-1} N as $NaNO_3$ and 20 ug L^{-1} P as K_2HPO_4.). We subsequently replenished 5% of this initial nutrient dose per day throughout the experiment. We inoculated all tanks with 50 mg dry weight of *Scenedesmus acutus* and let this algae grow for one week prior to introducing any hosts.

The experiment was conducted in two blocks: the case 1 genotype in 2009 and cases 2 and 3 in 2012. Both experiments crossed focal host genotype with presence/absence of the diluter and included diluter-only tanks. The 2012 experiment also included algae-only tanks. All treatments were replicated 4-6 times. In 2012, tanks were inoculated with low densities of focal hosts (S_{FH} = 15 L^{-1}) and allowed to increase in density for two weeks. Then, appropriate tanks were inoculated with equivalent densities of competitor/diluters ($S_{C/D}$= 100 L^{-1}) and allowed to increase in density for an additional two weeks. In 2009, tanks started with similar conditions to 2012. We used greater starting host densities than in the simulations because hosts in the simulations

approached their equilibria much more rapidly than in the experiment. In both experiments, epidemics were initiated with the addition of fungal spores after four weeks (Z = 5,000 L^{-1}). Host densities at this point corresponded qualitatively to host densities in simulations when spores were added (Fig. 2). We sampled one liter from each tank twice per week with 80 um mesh sieves. We tracked infected and uninfected host densities as well as infection prevalence through time (using microscopes to quantify samples and visually diagnose infections [50X]). Epidemics lasted 3-5 host generations, and approximately three parasite generations.

We quantified epidemics for each tank in our experiments by integrating the area under time series of infection prevalence and log-transformed density of infected hosts. Then, we compared epidemics with and without the competitor/diluters (and among focal host genotypes) with *t*-tests. Similarly, we quantified uninfected host density for each tank by integrating the area under the log-transformed density curves, and compared these quantities with integrated density *t*-tests. Visually, these tests compare the areas under the curves presented in Figs 2-4. Total host densities are also shown in Appendix S2 (Fig. S3). We also used *t*-tests to compare the density of diluters competing with our different focal hosts at the time of spore addition.

RESULTS

Overall, model predictions qualitatively matched experimental results (Figs. 2-4). We cannot test for block differences between the 2009 and 2012 mesocosm experiments. However, the agreement between parameterized model predictions and experimental results allows us to focus our argument on variation among the traits of our focal host genotypes. Our trait measurements revealed that the competitor/diluter was the superior competitor (lowest R^*: Fig. 1), and was thus predicted to outcompete all focal host genotypes over long periods of time. However, R^*'s were similar enough that

competitive replacement was slow (Grover 1997),and did not occur in any experiments. Indeed, our simulations predicted that competitive replacement ($S_{FH} < 1\ L^{-1}$) would only occur after 216 days of competition (~22-30 generations), even for our weakest competing focal host. With these points in mind, during the 31-35 days of our experimental epidemics, we show three trait-dependent outcomes of dilution among competing hosts: dilution failure (case 1), dilution success (case 2), and dilution ambiguity/irrelevance (case 3).

Case 1: Dilution failure (strong competitor, large epidemic)

The dilution effect failed for the focal host predicted to compete strongly (low R^*) and spread disease extensively (high R_0). When alone, these hosts drove large epidemics. Infection prevalence and infected density were both higher than the other focal host genotypes (Figs. 2 & 3 A,B; *t*-tests, all $p < 0.001$). Meanwhile, at the start of epidemics, diluters reached lower densities with this focal host than with the other two (Fig. 4 A,B; *t*-tests, both $p < 0.01$). Due in part to this competitive constraint, competitor/diluters failed to significantly reduce infection prevalence during epidemics (Fig. 2 A,B; *t*-test, $p > 0.3$). Most likely, competitor/diluters were not dense enough to inhibit disease by 'vacuuming' the large number of spores released by this focal host (Fig. 1 A). Although they marginally reduced the density of infected focal hosts, this effect was not statistically significant (Fig. 3 A,B; *t*-test, $p < 0.1$). Presence of competitor/diluters did lower mean densities of uninfected focal hosts (*t*-test, $p < 0.01$), although focal host populations crashed during epidemics regardless (Fig. 2 C,D). Finally, spillover from the large focal host epidemics even caused a small outbreak (i.e., amplified disease) in the diluter population (Fig. 2 A,B; *t*-test, $p < 0.05$). Thus, when focal hosts compete strongly and spread disease extensively, friendly competition can

produce a double failure: uncontrolled disease for focal hosts and spillover of disease into the competitor/diluters (i.e., an amplification effect).

Case 2: Dilution success (weak competitor, moderate epidemic)

The dilution effect succeeded for the focal host with weak competitive ability (high R^*) and moderate potential to spread disease (moderate R_0). When alone, these focal hosts drove intermediate epidemics (Figs. 2 & 3 C,D). Infection prevalence was lower than case 1 ('failure'; *t*-test, $p < 0.001$) and higher than case 3 ('ambiguity/irrelevance'; *t*-test, $p < 0.05$). Furthermore, density of infected hosts was lower than case 1 (*t*-test, $p < 0.0001$) and equivalent to case 3 (*t*-test, $p > 0.7$). At the start of epidemics, diluters reached higher density than in case 1 (Fig. 4 C,D; *t*-test, $p < 0.01$), but were equivalent to case 3 ($p > 0.7$). Because of the moderate epidemic size and their high density, diluters reduced both infection prevalence (Fig. 2 C,D, *t*-test, $p < 0.05$) and density of infected hosts (Fig. 3 C,D, *t*-test, $p < 0.01$) in the focal host population. No spillover was detected (*t*-test, $p > 0.5$). The model also predicted a small reduction in uninfected host density with competitor/diluters (especially relative to case 3; fig 3 C). However, this reduction was too small in the experiment for us to detect statistically (*t*-test, $p > 0.4$). Thus, for focal hosts with weak competitive ability and moderate R_0, the dilution effect succeeded with minimal density cost and no spillover (no amplification).

Case 3: Dilution ambiguity/irrelevance (weak competitor, small epidemic)

The presence of diluters had ambiguous effects (due to multiple definitions of 'disease risk') for focal hosts with weak competitive ability (high R^*) and low potential to spread disease (low R_0). Simulated epidemics spread very slowly, remaining below 1% infection prevalence (Fig. 2 E) and one infected host per liter (Fig. 3 E). In the

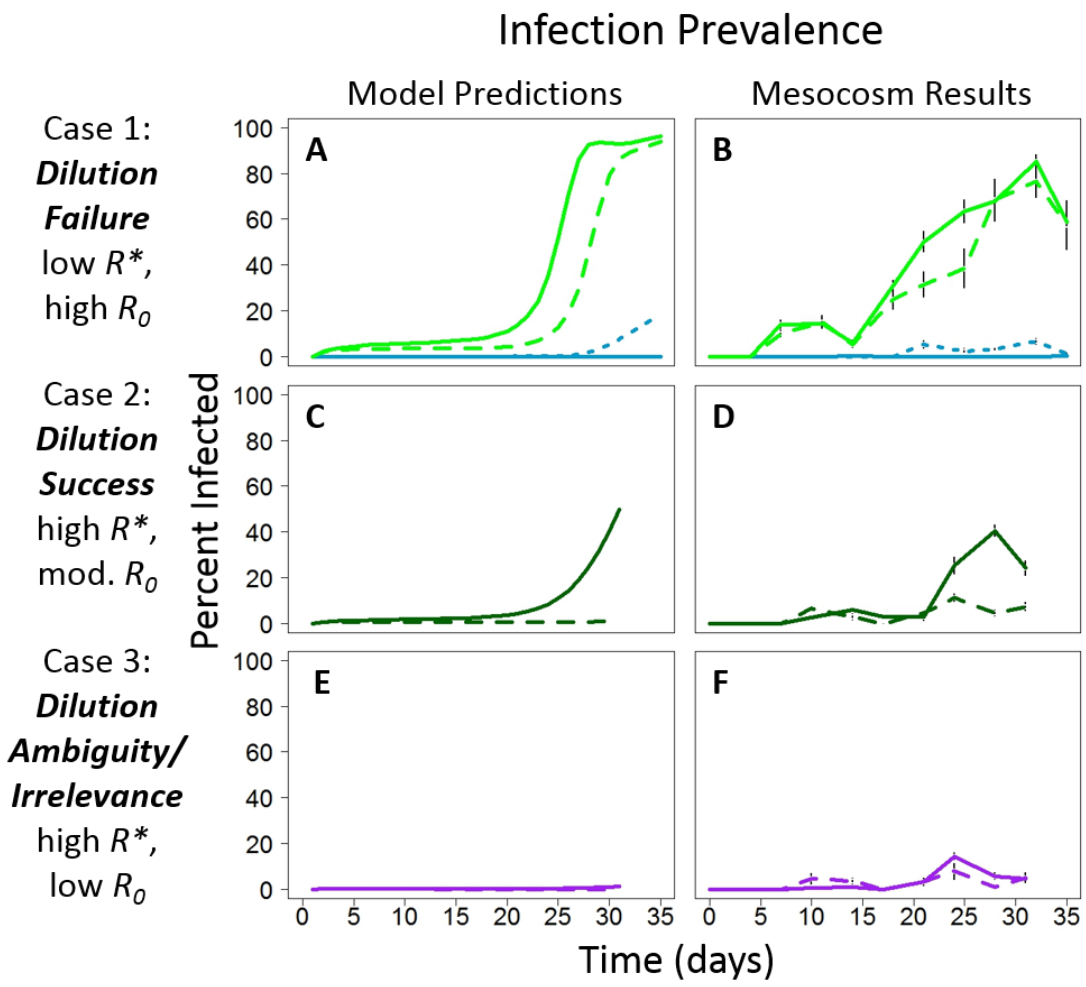

Figure 2. Variation in infection prevalence and the outcome of dilution depend on competitive ability (*R**) and the potential for disease spread (R_0) among three focal host genotypes. Parameterized model simulations (left column) qualitatively predict experimental results (right column). (A,B) Competitor/diluters ***fail*** to significantly reduce infection prevalence for focal hosts that compete strongly and spread disease extensively. Moreover, disease spills over into the diluter population, presenting an amplification effect. (C,D) Competitor/diluters ***succeed*** in significantly reducing infection prevalence for focal hosts that compete weakly and spread disease moderately. (E,F) Dilution is ***irrelevant*** (in terms of infection prevalence) for focal hosts that compete weakly and spread disease poorly. Solid lines: focal hosts alone; dashed lines: focal hosts with competitor/diluters; blue solid lines: competitor/diluters alone; blue dotted lines: competitor/diluters with focal hosts. Competitor/diluters shown only in (A,B). Error bars are standard errors.

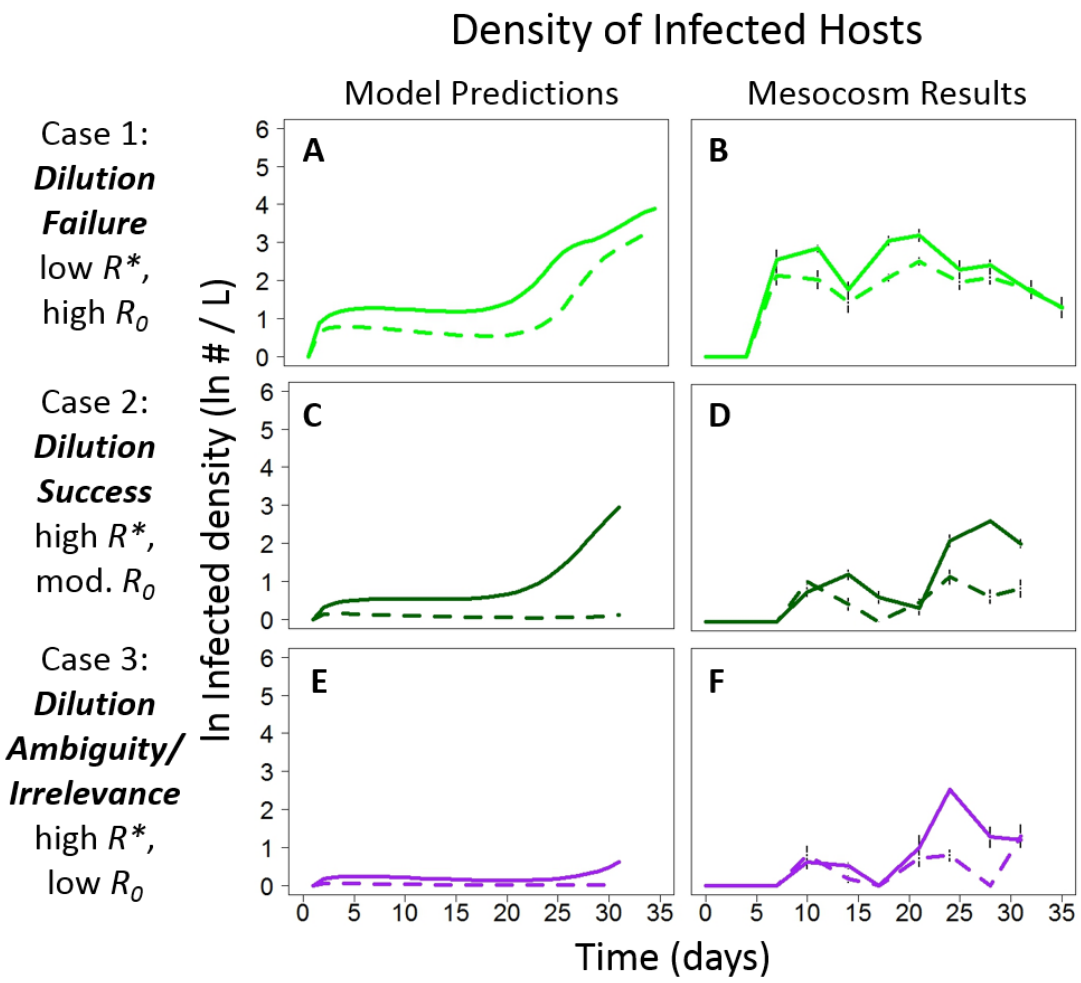

Figure 3. Variation in density of infected focal hosts depends on competitive ability (*R**) and the potential for disease spread (R_0) among three focal host genotypes. Parameterized model simulations (left column) qualitatively predict experimental results (right column). (A,B) Competitor/diluters **fail** to significantly reduce the density of infected focal hosts that compete strongly and spread disease extensively (although they do marginally reduce the density of these infected hosts). (C,D) Competitor/diluters **succeed** in reducing the density of infected focal hosts that compete weakly and spread disease moderately. (E,F) Competitor/diluters also succeed in reducing the density of focal hosts that compete weakly and spread disease poorly. However, competitor/diluters were irrelevant in terms of infection prevalence for this host (Fig. 3); thus, dilution is **ambiguous**. Solid lines: focal hosts alone; dashed lines: focal hosts with competitor/diluters. Error bars are standard errors.

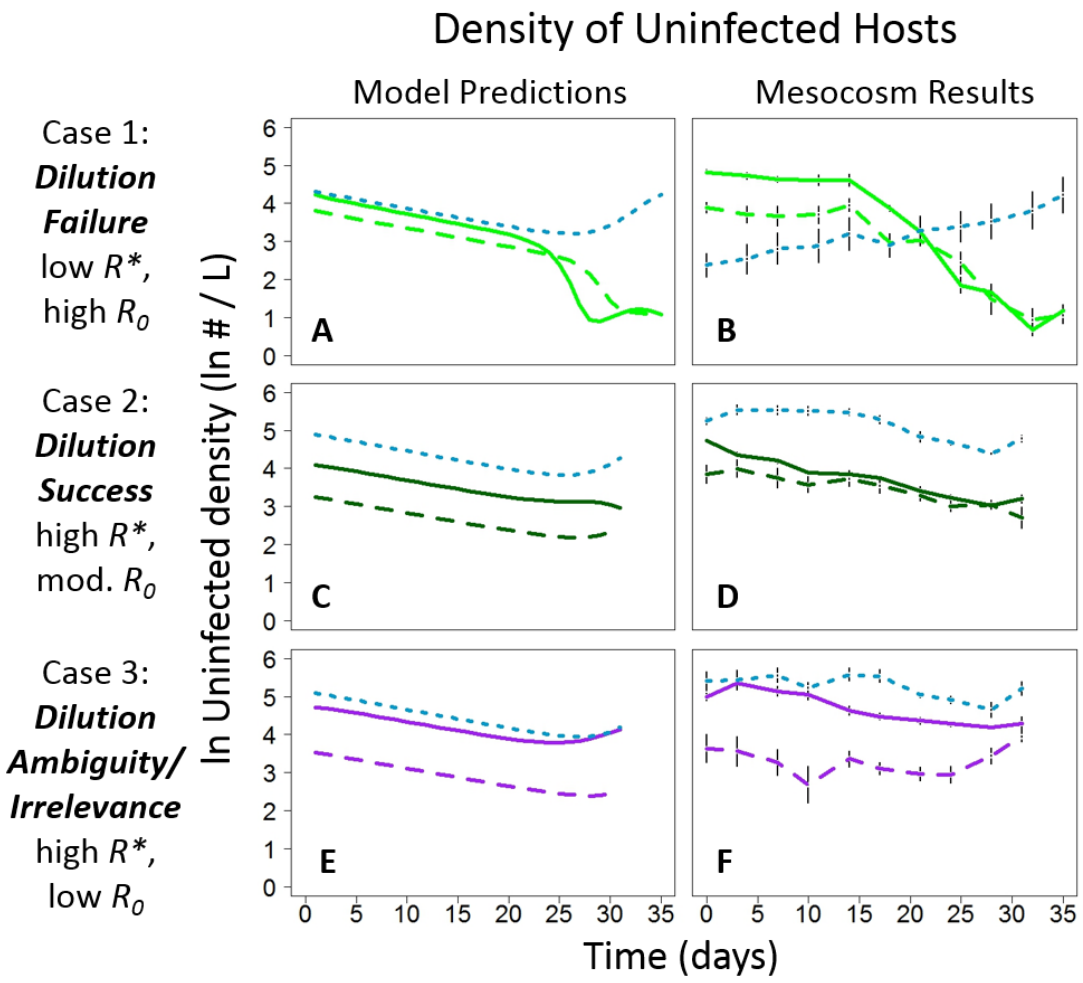

Figure 4. Variation in density of uninfected (susceptible) focal hosts and competitor/diluters depends on competitive ability (*R**) and the potential for disease spread (R_0) among three focal host genotypes. Parameterized model simulations (left column) qualitatively predict experimental results (right column). Competitor/diluters significantly reduce the density of (A,B) uninfected focal hosts that compete strongly and spread disease extensively and (E,F) focal hosts that compete weakly and spread disease poorly. (C,D) Density of uninfected focal hosts that compete weakly but spread disease moderately is unaffected by competitor/diluters. Competitor/diluter density prior to the epidemic is lower when competing with (A,B) the strong-competitor focal host than when competing with (C-F) the two weak-competitor focal hosts. Solid lines: focal hosts alone; dashed lines: focal hosts with competitor/diluters; blue dotted lines: competitor/diluters with focal hosts. Error bars are standard errors.

experiment, infection prevalence was lower for this host alone than in case 1 ('failure'; *t*-test, $p < 0.001$) and case 2 ('success'; *t*-test, $p < 0.05$) (Fig. 2 F). Density of infected hosts was also lower for this host alone than in case 1 (*t*-test, $p < 0.0001$), but not case 2 (*t*-test, $p > 0.1$) (Fig. 3 F). The model did not predict this detail (Fig. 3 E). Competitor/diluters did not significantly reduce infection prevalence in this focal host (Fig. 2 F; *t*-test, $p > 0.7$), likely because competitor/diluters were nearly as good at spreading disease as these low-R_0 focal hosts (similar R_0's: Fig. 1 E). Thus, diluters were irrelevant in terms of infection prevalence, despite reaching densities similar to case 2 (Fig. 4 F; *t*-test, $p > 0.7$). With so little disease, spillover (i.e. amplification) was neither predicted nor detected (*t*-test, $p > 0.5$). Nevertheless, competitor/diluters did significantly reduce density of infected focal hosts during the epidemic (Fig. 3 F; *t*-test, $p < 0.05$). This effect was likely driven by the competitive interaction between host species rather than vacuuming, since infection prevalence was not significantly different between treatments ($p > 0.7$). Indeed, competitor/diluters vastly outnumbered this focal host overall, and uninfected focal host density was also strongly reduced by competition (Fig. 4 E,F; *t*-test, $p < 0.05$). For focal hosts with these traits, the outcome of dilution is ambiguous and depends on the definition of disease risk (infection prevalence versus density of infected hosts). From a density perspective, dilution was successful. However, from a prevalence perspective, dilution was irrelevant.

DISCUSSION

Our three case studies mathematically predicted and experimentally confirmed three qualitatively different outcomes of the friendly competition module. To predict these differences, we mechanistically linked competition, disease spread, and outbreak size (both in terms of prevalence and number of infected hosts). More specifically, the outcome of dilution among competitors—success, failure, or ambiguity/irrelevance—

depended predictably on encounter reduction (i.e. vacuuming), host regulation (i.e. the strength of competition, R^*), and the magnitude of disease spread (indexed by R_0). In case 1, the focal host genotype was a strong competitor (low R^*) and a strong spreader of disease (high R_0). The dilution effect failed for this focal host, because competition constrained the diluter population (limiting vacuuming and constraints on the focal host), while large epidemics overwhelmed diluters with infective spores. Disease even spread to competitor/diluters via spillover from the focal host epidemic (i.e., an amplification effect). In case 2, the focal host genotype was a weak competitor (high R^*) and a moderate spreader of disease (moderate R_0). The dilution effect succeeded here, because more diluters (i.e., stronger host regulation) sufficiently vacuumed the moderate density of infective spores. In case 3, the focal host genotype was a weak competitor (high R*) and a weak spreader of disease (R_0). Here, the dilution outcome became ambiguous, because competitor/diluters significantly lowered the density of infected focal hosts but were irrelevant regarding infection prevalence (because prevalence was so low). These three case studies emphasize the range of dilution outcomes (success, failure, and ambiguity/irrelevance) that can occur even within a simple community module (Bolnick *et al.* 2011). Yet, using measured traits of our hosts as a mechanistic guide, we have explained—and even predicted—these seemingly idiosyncratic outcomes (e.g., Salkeld *et al.* 2013).

Our dynamical model and multi-generational experiments enabled novel synthesis of encounter reduction and host regulation (but see Keesing *et al.* 2006; Johnson *et al.* 2008; Johnson *et al.* 2012a; Wojdak *et al.* 2014). These dilution mechanisms do not act independently, for two reasons. First, competition between focal hosts and diluters determines regulation of focal hosts (potentially reducing net disease spread), and also the magnitude of the net vacuuming (encounter reduction) provided by the competitor/diluters. Net release of infective spores is the product of infected focal

host density and their per-capita spore yield (Fig. 1). Likewise, 'net vacuuming' is the product of competitor/diluter density and their per-capita vacuuming rate. Focal hosts which compete strongly (case 1) do not receive the disease-mediating benefits of either strong regulation or strong net vacuuming. Weaker competitors (cases 2 and 3) experience some combination of stronger regulation *and* higher net vacuuming. Thus, the outcomes of dilution over multiple host generations could hinge sensitively on relatively small differences in competitive ability.

A second dilution mechanism interaction, density-mediated feedbacks, also likely contributed to the outcomes in our model and experiment. Consider, for example, the following hypothetical four-step feedback cycle: 1) Disease outbreaks kill focal hosts. 2) As hosts die, diluters are released from competition and increase in density. 3) A higher density of diluters enhances their net vacuuming rate. 4) Higher net vacuuming reduces disease spread and prevents focal hosts from dying. We cannot directly track this four-step process in our model and experiments, because all four steps are occur simultaneously. Therefore, we cannot fully disentangle the effects of host regulation and encounter reduction. However, our model and experiments suggest that the net outcome of this feedback cycle likely depends on traits of the interacting species: their relative competitive abilities, diluters' per-capita vacuuming rate, and the ability of focal hosts to spread disease. These feedbacks cannot occur in experiments that only last a single host generation, even though they likely operate in host communities in nature. Thus, these dynamics need to become part of the conceptual repertoire for the dilution effect.

The competition component of our 'friendly competition' model may unify some existing theory for dilution. Competition in extant dilution theory has been modeled as an interaction coefficient among hosts (Schmidt & Ostfeld 2001), the effect that a diluter species has on overall species density (Rudolf & Antonovics 2005; Ogden & Tsao 2009),

and how host density scales with richness (Roche *et al.* 2012; Mihaljevic *et al.* 2014). These various modelling forms and assumptions have obscured the recurrent role that competition has played in the dilution effect literature. Simultaneously and independently however, they have emphasized the importance of competition in modulating the dilution effect. Model assumptions (e.g., specifically *how* host richness scales with density) can fundamentally change whether or not a dilution effect is predicted (Rudolf & Antonovics 2005; Ogden & Tsao 2009; Mihaljevic *et al.* 2014). This result is synonymous with ours: the outcome of dilution can hinge on the strength of competition among host species. We argue that parameterized resource competition (either explicit or phenomenological) is a preferable, clear alternative to cryptic and weighty model assumptions about the densities of interacting species. Parameterized competition can mechanistically determine—as an outcome, not an assumption—the strength of competition and its importance for dilution.

Likewise, we argue that competition (manifested as host densities) is an important design component in experiments that test for dilution effects. Competition among hosts is a prominent feature in empirical plant and animal dilution systems (Mitchell *et al.* 2002; Johnson *et al.* 2008; Clay *et al.* 2009; Hall *et al.* 2009a; Johnson *et al.* 2009; Johnson & Thieltges 2010; Becker *et al.* 2014; Lacroix *et al.* 2014; Rottstock *et al.* 2014). Substitutive experimental designs are most appropriate when hosts compete strongly, thus reducing disease (e.g. Mitchell *et al.* 2002; Rottstock *et al.* 2014). Especially in single generation experiments, the strength of host regulation is artificially imposed (via densities of hosts in the experimental design). Substitutive designs can confound host regulation with other mechanisms (e.g., encounter reduction), and artificially strong host regulation could overshadow the relevant mechanisms that reduce disease in nature. Great care must therefore be taken to ensure that experimental densities reasonably resemble natural communities. Designs that manipulate both host

density and community composition can decouple the effects of host regulation and encounter reduction (Johnson *et al.* 2008; Wojdak *et al.* 2014). However, these designs still obscure the dynamical feedbacks and interactions described above. Thus, we urge more experimental tests of dilution theory that incorporate multi-generational competition (e.g., Mitchell *et al.* 2002; Johnson *et al.* 2012a; Rottstock *et al.* 2014).

Focusing on density of infected hosts versus infection prevalence might change the interpretation of friendly competition here. For instance, competitor/diluters reduced infection *prevalence* in only one of our case studies. However, they reduced *density* of infected focal hosts in two of our three case studies (and marginally reduced it in the third). Such a density-focused outcome might herald unequivocal success in systems involving wildlife reservoirs of human disease, such as Schistosomiasis (Johnson *et al.* 2009) and Hanta virus (Clay *et al.* 2009; Suzan *et al.* 2009). In these systems, reduced density of wildlife hosts infected with human parasites would signal a favorable outcome of dilution, as long as there is no compensatory *increase* in infection prevalence (e.g. Ogden & Tsao 2009). Case 3 ('ambiguity/irrelevance') would be a success under these criteria. However, this same outcome (reduced density of infected *and* susceptible hosts) might prove too costly for wildlife diseases like amphibian chytrid (Bd; Venesky *et al.* 2014) and trematode infections (Ribeiroria; Johnson *et al.* 2013), or in agriculture (Boudreau 2013). For such hosts of economic or conservation concern, the regulatory component of friendly competition may unacceptably depress density of uninfected hosts, even if competitor/diluters do reduce infection prevalence (as in case 2, 'success'). Thus, the costs and benefits of friendly competition depend sharply on perspective (i.e., from human disease control vs. conservation/agriculture). Unless we clearly define our definition of 'dilution success' on a case-by-case basis, this ambiguity could clearly propagate more confusion in the dilution effect literature.

Our results also prompt a set of questions best framed over broader parameter space, temporal scale, and spatial scales. First, a thorough mathematical analysis of our model would allow us to freely manipulate traits, eliminating the constraints of our three guiding empirical case studies (e.g., Cáceres *et al.* 2014). We could analyze the sensitivity of friendly competition's outcomes to variation in each host trait independently, and use our inferences to better disentangle the effects of host regulation and encounter reduction. Second, as parameterized in the model, our competitor/diluter can outcompete all focal hosts over long enough time periods. Theory for long-term dynamics of friendly competition therefore require better representation of species niches that could promote coexistence between focal hosts and competitor/diluters. After all, these two hosts do coexist in nature (Tessier & Woodruff 2002; Hall *et al.* 2010b). Third, such a realistic long-term theory may require embracing evolutionary changes in hosts. Both competition (Pimentel 1968) and disease (Duffy *et al.* 2012) can drive rapid evolutionary changes in genetically diverse host populations; however it is unclear how selection could regulate friendly competition and dilution through feedbacks (e.g., if all three of our focal host genotypes occurred together in a genetically diverse population). Fourth, armed with explicit dilution models, community ecologists could expand friendly competition to larger spatial scales. Do competition-colonization tradeoffs (Tilman 1990) or life history-pathogen defense tradeoffs (Johnson *et al.* 2012b) link traits that both regulate local dilution *and* determine regional assembly of host communities? All four of these extensions (parameter space exploration, coexistence, evolution, and community assembly) require estimating the variation and covariation of host and diluter phenotypes in nature. With these data, we could search for traits that promote host coexistence, aid in dispersal and persistence among sites, and determine competitive ability (R^*) and the potential for disease spread (R_0). Insight into the variances and covariances among these traits in focal hosts and diluters in nature could

ultimately catalyze a mechanistic eco-evolutionary framework for the dilution effect across landscapes and through ecological time.

Even without these extensions, friendly competition speaks to some immediate conservation and disease management concerns. For instance, when hosts compete for resources (e.g., Becker *et al.* 2014; Lacroix *et al.* 2014), reintroduction of diluters to control disease could exact an undesirable cost on density of focal hosts. Alternatively, diluters constrained by competition might fail to control disease in hosts that drive severe epidemics. In extreme cases of failure, diluters could even suffer disease, via spillover/amplification themselves. These hazards prompt precise delineation of potential future goals for disease management using the dilution effect. Sometimes, the goal might center on boosting density of healthy focal hosts (e.g., in threatening wildlife diseases like amphibian chytridiomycosis: Becker *et al.* 2014; Venesky *et al.* 2014). In these cases, management decisions must balance the inherent cost of competition with the potential benefit of reduced disease. Alternatively, human disease control efforts (e.g., for Hanta virus: Clay *et al.* 2009; Suzan *et al.* 2009) may warrant great reductions of the density of focal hosts through competition with diluters. In these instances, the inherent cost of competition from diluters might reap management benefits. All of these possibilities arise because local species interactions can potentially interfere with disease transmission but exact other ecological consequences. Thus, a more tested, dynamical, and mechanistic theory will push the dilution effect beyond its phenomenological foundation and help us better anticipate its success, failures, ambiguity, or irrelevance.

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CHAPTER 2 SUPPORTING INFORMATION

Appendix S1

In this Supplementary Appendix, we provide more details for estimates of the traits (parameters) in the mathematical model for 'friendly competition' (described in Box 1). More specifically, we outline the methodological and statistical details for foraging rate (f), per-spore susceptibility (u), conversion efficiency (e), virulence (v), background death rate (d), and spore yield as a function of resources, $\sigma(R)$. We also describe the estimation of the synthetic indices of competitive ability (R^*) and disease spread (R_0). Finally, we include two supplementary figures, displaying variation in virulence among genotypes (Supplementary Figure 1) and spore yield as a function of resources (Supplementary Figure 2).

Trait measurement assays

We estimated genotype-specific foraging rate (f), conversion efficiency (e), per-spore susceptibility (u), virulence (v), and spore yield (σ) with data collected from foraging assays, infection assays, and life table experiments. Note that below, we drop the genotype subscript i used in Box 1, for clarity of notation. All focal host and diluter genotypes were grown in cultures at 20°C and fed high quality laboratory-cultured algae daily (1.0 mg L^{-1} dry weight of *Ankistrodesmus falcatus*, reared in WC media). Cultures were maintained in filtered (Pall A/E: 1.0 µm) lake water under ideal conditions for three generations in order to minimize any maternal affects. We then collected neonates and reared them for five days under ideal conditions. All six-day old animals were then placed in the life table experiment; a subset of these were also used for the infection assay, and a further subset of these were also used in the foraging assay.

Estimation of foraging (exposure) rate (*f*)

Foraging assay: We calculated foraging rate by comparing fluorescence of ungrazed and grazed algae (Sarnelle & Wilson 2008). We transferred 20 six-day old animals of each genotype individually into culture tubes containing 20 mL of filtered lake water and 1.0 mg L^{-1} dry weight *A. falcatus*. We also included eight tubes with algae but without hosts (to serve as ungrazed controls). All tubes were placed in a tube rotator, which continuously resuspended algae. Hosts were allowed to graze at 20° C for 22 hours in complete darkness. After 22 hours, hosts were transferred into fresh 50 mL tubes as part of the life table experiment. We used in vivo fluorimetry to calculate relative fluorescence of media from all culture tubes (using a Turner Trilogy Laboratory Fluorometer).

Parameter estimation: Foraging rate (*f*) is derived by fitting a simplified version of the "resource dynamics" equation (Box 1). Because we conducted our foraging rate assay in darkness, we assume no growth of algae occurred. Additionally, with only susceptible hosts (*S*) grazing, our differential equation simplifies to:

$$\frac{dR}{dt} = -fRS \qquad \text{eq. S1}$$

Solving this exponential equation for resource density (*R*) yields:

$$R_{rem} = R_{init} \exp(-fSt) \qquad \text{eq. S2}$$

where R_{rem} is the remaining resource, R_{init} is the initial resource (at time $t = 0$), and t is the duration of the trial (22 hours). Solving for *f*, then:

$$f = \ln\left(\frac{R_{init}}{R_{rem}}\right)\frac{V}{t} \qquad \text{eq. S3}$$

where 1/*S* was replaced with experimental volume (*V*). We bootstrapped 95% confidence intervals for genotype-specific foraging rates with 10,000 iterations in R (R Development Core Team 2008).

Estimation of per-spore susceptibility (*u*)

Infection assay: All individuals in the foraging assay plus an additional 40 individuals from each genotype (excluding the case 1 focal host genotype for logistical constraints) were used in the infection assay (see Hall *et al.* 2010). We exposed individual hosts to infective spores (100 spores ml^{-1} or 450 spores ml^{-1}, for 22 hours). Infection assay animals that were not part of the foraging assay were treated identically (transferred into culture tubes with 20 mL of filtered lake water and allowed to graze 1.0 mg L^{-1} dry weight *A. falcatus* for 22 hours), but were inverted every half hour instead of being placed in the tube rotator. Replication at each spore density was planned strategically based on prior knowledge of each genotype's susceptibility (Hall *et al.* 2010). After 22 hours of exposure, hosts were transferred into fresh 50 mL tubes as part of the life table experiment. We then visually diagnosed infection while monitoring the life table experiment.

Parameter estimation: We estimated per-spore susceptibility (*u*) by fitting a simplified version of the "susceptible host dynamics" equation (Box 1). By focusing only on the loss of susceptible hosts due to transmission and defining the transmission coefficient as the product of exposure and per-spore susceptibility ($\beta = u\,f$), we arrive at:

$$\frac{dS}{dt} = -\beta Z S \qquad \text{eq. S4}$$

Solving this equation for remaining susceptible hosts after exposure time *t* yields S_{rem}:

$$S_{rem} = S_{init}\,\exp(-\beta Z t) \qquad \text{eq. S5}$$

where S_{init} is the initial number of hosts in the experiment. We estimated this β using maximum likelihood and the BBMLE package in R, with our binary infection assay data as S_{rem} and the binomial distribution serving as the likelihood function. We generated 95% confidence intervals around β by bootstrapping our infection data with 10,000

iterations. We then calculated per-spore susceptibility u (hosts spore^{-1}) as $u = \beta / f$. We generated 95% confidence intervals around u by bootstrapping values of β / f.

Estimation of conversion efficiency (*e*) and virulence (*v*)

Life table experiment: All individuals in the infection assay plus an additional 10 individuals from each genotype were used in a life table experiment to estimate conversion efficiency (*e*) and virulence (*v*) (see Hall *et al.* 2010). These 10 new individuals were treated identically on day 6 but were not exposed to spores. Thereafter, all animals were transferred daily into 50 mL tubes with fresh filtered lake water and 1.0 mg L^{-1} dry weight *A. falcatus*. Each day we counted and removed neonates and recorded host deaths until all infected hosts died (17 days). Infected hosts were isolated in 0.25 mL lake water upon death.

Note that overall births in our model (Box 1) are the product of total host density ($S+I$), resource density (R), foraging rate (f), and conversion efficiency (e). Although our life table experiment allows us to measure instantaneous birth rate (b) directly, we need to decouple this estimate of birth rate from the other terms in our model (i.e., foraging rate (f) and resource density (R)) that contribute to instantaneous birth rate (b). We accomplish this by estimating the conversion efficiency parameter (e), which is essentially births per food consumed.

Parameter estimation (e): We estimated conversion efficiency (e) as birth rate of uninfected hosts (b) per algae consumed:

$$e = \frac{b}{fR} \qquad \text{eq. S6}$$

where R was measured in µg L^{-1} of ethanol-extracted chlorophyll *a*). Estimation of the birth rate parameter requires summing instantaneous per capita population growth rate

(*r*) plus background mortality rate (*d*); i.e., $b = r + d$. To estimate *r*, we solved the standard Euler-Lotka equation:

$$1 = \sum_t \exp(-rt)\, l_t F_t \qquad \text{eq. S7}$$

Here, l_t is the proportion of animals surviving to day *t* and F_t is the average fecundity on day *t*. We estimated death rate (*d*) by assuming that time until death followed an exponential distribution. This distribution provides the likelihood (ℓ) of constant death rate (*d*) given the time-until-death data for each host (t_d):

$$\ell(d|t_d) = d \exp(-dt_d) \qquad \text{eq. S8}$$

Since not all hosts died by the end of the experiment, we included the "censored" observations using the likelihood that the animal survived at least to the end of the experiment, t_e = 23 days:

$$\ell(d|t_d > t_e) = \exp(-dt_e) \qquad \text{eq. S9}$$

We found the maximum likelihood estimate of *d* by minimizing the sum of these negative log-transformed likelihoods (eqs. 8 and 9). With our *r* and *d* estimates, we bootstrapped 95% confidence intervals for genotype-specific birth rates. Then, with estimates of *f*, *R*, and *b*, we calculated *e* and bootstrapped confidence intervals around it.

Parameter estimation (v): We estimated virulence (*v*) as the difference in death rate between infected and uninfected hosts from the life table experiment. We estimated background death rate (*d*) of uninfected hosts as described above (eqs. 8 and 9). We calculated overall death rate of infected hosts ($d + v$) using the same equations. Then we calculated virulence (*v*) as the difference and bootstrapped confidence intervals around it (Fig. S1).

Parameter estimation (d): Although estimating virulence and birth rate required estimating background death rate, *d*, our life table experiment was not designed to provide precise and accurate estimates for *d*. We would need a longer experiment that would allow more uninfected hosts to die naturally. As such, our current parameters

likely underestimate *d*. Because of this uncertainty in *d*, we use a reasonable estimate (0.05 day^{-1}) in all of our simulations (Hite *et al.* unpublished manuscript). Thus, variation in other traits (with better estimates) drives qualitative differences in synthetic indices and simulations.

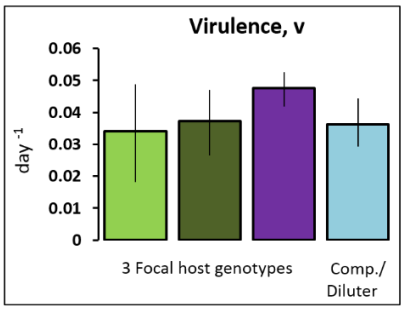

Figure S1. Variation in increased death due to virulence. Because our life table experiment was not long enough to reliably estimate background death rate *d*, (not enough hosts died of natural causes), we used *d* = 0.05 day^{-1} in all simulations. However, we estimated virulence *v* (increased death rate due to infection) for each genotype because infected hosts all died within two weeks of the life table experiment. Death rate of infected hosts was then *d*+*v*. Error bars are 95% confidence intervals.

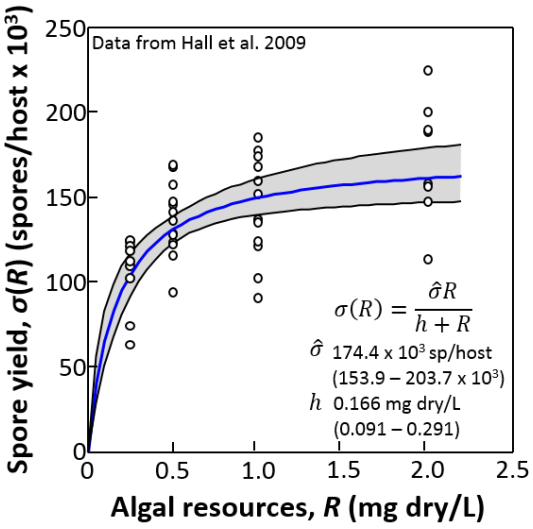

Figure S2. A functional form for the increase of spore yield with density of algal resources, *σ(R)*, using previously published data (Hall *et al.* 2009). We used the half saturation constant (*h*) of the type II function to then map spore yield to resources for the focal hosts here (after estimating maximal spore yield, $\hat{\sigma}$, for each clone and converting *h* into appropriate chl-*a* units). Parameter estimates are given with bootstrapped 95% confidence intervals; the grey 95% envelope was also bootstrapped.

Estimation of spore yield (σ) as a function of resources (R)

Although spore yield (σ) increases with resources (R), we only measured spore yield at one resource level (using dead infected hosts from the life table experiment). We ground these hosts with an automatic pestle, counted their spores with a haemocytometer, and bootstrapped 95% confidence intervals for genotype-specific spore yields (Fig. 1 A). Then, we fit a function to pre-existing data (Hall *et al.* 2009) to describe how spore yield increases with resources in our model (Box 1):

$$\sigma(R) = \frac{\hat{\sigma} R}{h+R} \qquad \text{eq. S10}$$

Here, h is the half saturation constant of spores growing within a host, R is the current concentration of resources (µg L^{-1} chl-*a*), and $\hat{\sigma}$ is the maximum spore yield for a genotype. The half saturation constant h was fitted to a single host genotype with spore yields estimated at four resource concentrations (Hall *et al.* 2009) (see Fig. S2 for data and confidence intervals). Then, we converted the units of h from mg L^{-1} dry weight to µg L^{-1} chl-*a*, using a regression of algal dry weight versus fluorescence (not shown). We estimated maximum spore yield ($\hat{\sigma}$) for each genotype as:

$$\hat{\sigma} = \frac{\sigma(h+R)}{R} \qquad \text{eq. S11}$$

Here, σ is the average spore yield measured from the life table (Fig. 1 A), R is the concentration of resources used in the life table (measured in µg L^{-1} of ethanol-extracted chlorophyll *a*), and h

is the fitted half saturation constant (Hall *et al.* 2009) (Fig. 2).

Other parameters: algal growth rate (r) and carrying capacity (K); spore loss rate (m)

The estimate for algal K (250 µg L^{-1} chlorophyll-*a*) was the average ethanol-extracted chlorophyll-*a* from algae-only mesocosm tanks over the epidemic period

(measured with a fluorometer; not shown). We assumed a reasonable estimate for maximal algal growth rate (r = 0.9 day^{-1}) (Sterner & Elser 2002). Our estimates for spore loss rate m (0.2 day^{-1}) was based on prior knowledge of our study system (Civitello *et al.* 2013).

Synthetic indices of competitive ability (R^*) and disease spread (R_0)

For each clonal genotype of the focal host and for the competitor/diluter, we derived R^* by solving for the disease-free boundary equilibrium of our model (Box 1):

$$R^* = \frac{d}{ef} \qquad \text{eq. S12}$$

We derived R_0 using the next generation matrix approach, yielding:

$$R_0 = \frac{(fuS_b^*)\sigma(R^*)}{m+(fS_b^*)} \qquad \text{eq. S13}$$

which is the ratio of gains from infection (fuS_b^*) and spore release ($\sigma(R^*)$, following eq. S11), in the numerator, to losses of spores from spore mortality (m) and consumption (fS_b^*) (in the denominator). Here, S_b^* is equilibrial host density at the disease-free boundary equilibrium:

$$S_b^* = \frac{r}{f}\left(1 - \frac{R^*}{K}\right) \qquad \text{eq. S14}$$

where K is the carrying capacity of the algal resource (without hosts), r is maximal growth rate of the algal resource, and R^* is the minimal resource requirement of the focal host or competitor/diluter (eq. 12). We generated 95% confidence intervals around R^* and R_0 by bootstrapping over variation in all of the parameters involved (f, u, e, and σ).

Appendix S2

In this Supplementary Appendix, we display an additional figure showing the total host densities in all simulations and mesocosm experiments.

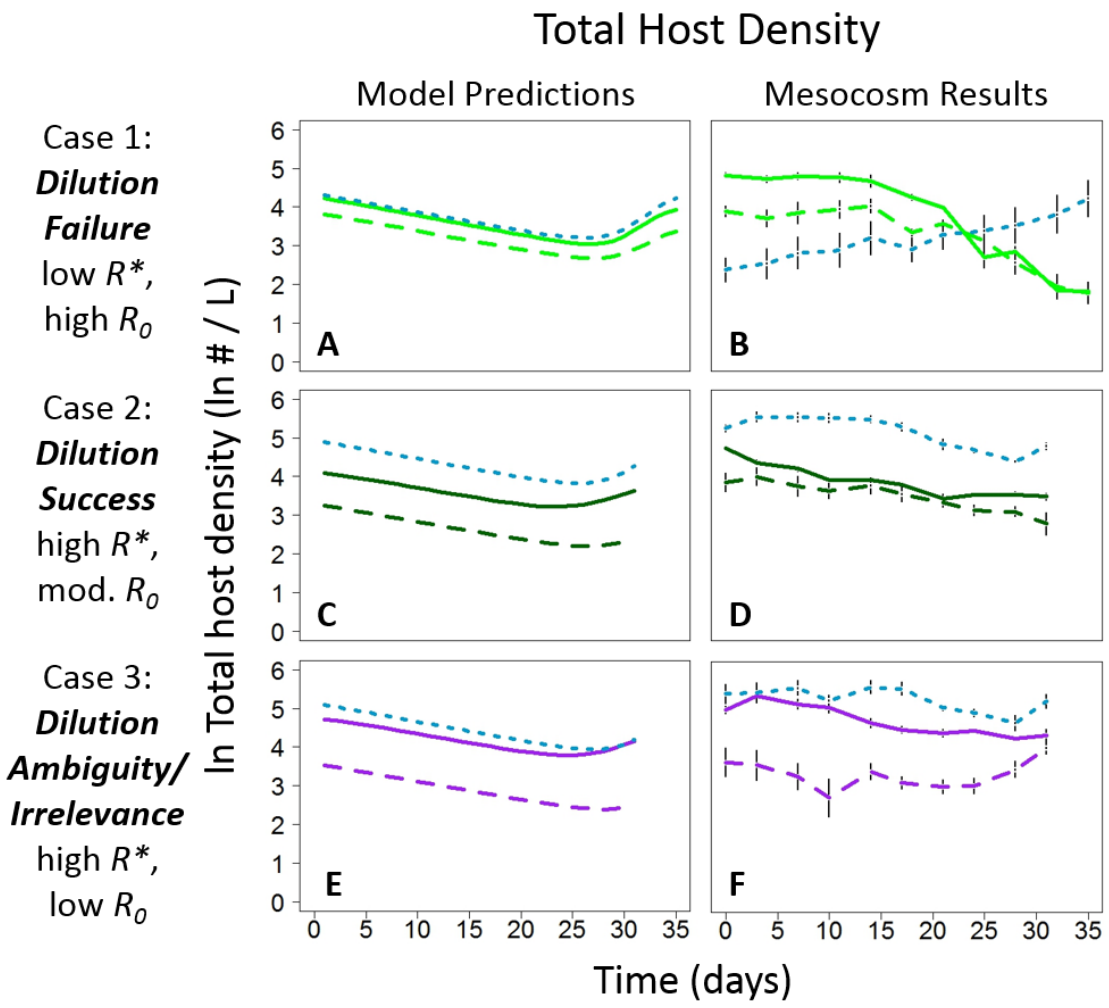

Figure S3. Variation in density of total (susceptible and infected) focal hosts and competitor/diluters depends on competitive ability (R^*) and the potential for disease spread (R_0) among three focal host genotypes. Solid lines: focal hosts alone; dashed lines: focal hosts with competitor/diluters; blue dotted lines: competitor/diluters with focal hosts. Error bars are standard errors.

LITERATURE CITED (CHAPTER 2 SUPPORTING INFORMATION)

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Chapter 3

When and how diluters reduce disease:

Host traits predict experimental outcomes of friendly competition

Citation:

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CHAPTER 3 ABSTRACT

The dilution effect is grounded in mechanistic species interactions, especially host regulation and encounter reduction. Yet few experiments delineate when and how these dilution mechanisms reduce infection prevalence and density of infected hosts. Here, we take a traits-based approach to predict disease outcomes when host regulation and encounter reduction operate together (i.e., in friendly competition). Among eight focal host genotypes, we measured two key traits and tracked their densities and infections in a mesocosm experiment (with and without competitor/diluters). Both focal host traits simultaneously regulated disease. Focal hosts benefited most from disease dilution when they experienced high disease risk (creating an opportunity for dilution) and competed weakly (allowing more competitor/diluters). However, competitor/diluters regulated each metric of disease through a different pathway: encounter reduction lowered infection prevalence, but host regulation reduced density of infected hosts. These insights may help predict how and when diluters reduce each metric of disease in a variety of disease systems.

INTRODUCTION

The dilution effect is an emerging controversial pattern in disease ecology (Keesing *et al.* 2006; Ostfeld & Keesing 2012; Johnson *et al.* 2015). It links decreases in species diversity with increases in disease risk for a focal host species. This pattern occurs when 'diluter' taxa interfere with transmission among more competent focal hosts, and these 'diluters' are lost from communities as diversity declines. Hence, loss of diversity (specifically, loss of diluters) can elevate disease risk (e.g., Ostfeld & Keesing 2000; Johnson *et al.* 2013; Lacroix *et al.* 2014). However, critics question the dilution effect's generality, sensitivity to different definitions of "diversity" and "disease risk," and the spatial scale under consideration (Randolph & Dobson 2012; Wood & Lafferty 2013; Wood *et al.* 2014). Additionally, apparent effects of diversity can actually be driven by correlational changes in focal host density (see Begon 2008) or frequency of key diluters (e.g., Strauss *et al.* 2016). These critiques propagate, in part, because of unclear mechanisms specifying *why* diversity correlates with disease. Nevertheless, diversity does appear to broadly inhibit parasites infecting both humans and wildlife (meta-analysis: Civitello *et al.* 2015). Thus, especially considering its many critiques, better mechanistic insight is needed for dilution effect theory. Specifically, this mechanistic focus must clearly delineate when and how diversity impacts disease (Ostfeld & Keesing 2012; Johnson *et al.* 2015).

Modules of interacting species can mechanistically link diversity and disease (e.g., Strauss *et al.* 2015). After all, a dilution effect ultimately results from interactions among focal hosts, parasites, and diluters (Keesing *et al.* 2006; Johnson *et al.* 2015). Yet the dilution mechanisms resulting from these interactions remain surprisingly understudied. One mechanism features diluters that *reduce encounters* between focal hosts and parasites. For parasites transmitted environmentally, encounter reduction

occurs when resistant diluters consume free-living parasites (Johnson *et al.* 2010; Civitello *et al.* 2013). Diluters can also *regulate* focal host populations via competition (Keesing *et al.* 2006). All else equal, lower focal host density reduces density-dependent transmission (Anderson & May 1981). These two mechanisms (host regulation and encounter reduction) combine in the 'friendly competition' module (Hall *et al.* 2009; Strauss *et al.* 2015). Although competition typically depresses fitness of both species, potentially 'friendly' competitor/diluters might net-benefit focal hosts by reducing disease. Potential friendly competition scenarios include rodents and hantavirus (Clay *et al.* 2009), snails and *Schistosoma* (Johnson *et al.* 2009), invaded intertidal communities (Johnson & Thieltges 2010), emerging amphibian diseases (Johnson *et al.* 2013; Venesky *et al.* 2014), and plant communities with fungal and viral pathogens (Mitchell *et al.* 2002; Boudreau 2013; Lacroix *et al.* 2014). Thus, friendly competition offers a framework to study two general dilution mechanisms that frequently operate together.

However, competitor/diluters do not always reduce disease. Instead, variation in focal host traits may regulate outcomes of friendly competition (Cáceres *et al.* 2014), as demonstrated by three empirical case studies and parameterized model simulations (Strauss *et al.* 2015). First, when focal hosts suffer high disease risk and compete strongly, competitor/diluters fail to reduce infection prevalence or density of infected hosts. These competitor/diluters likely become outcompeted, outnumbered by focal hosts, and overwhelmed by large epidemics. Second, when focal hosts experience moderate disease risk and compete weakly, competitor/diluters (which are more numerous) successfully reduce prevalence and density of infected hosts. Third, when focal hosts experience low disease risk and compete weakly, competitor/diluters reduce density but not prevalence of infections. Because these focal hosts resist disease nearly as strongly as competitor/diluters, opportunities for dilution likely disappear. These

divergent impacts on each metric of disease might also imply that different mechanisms regulate infection prevalence vs. density of infected hosts. However, overall among these three case studies (Strauss *et al.* 2015), both disease risk and competitive ability seem to be linked to competitor/diluters' impacts on disease.

Yet a fully mechanistic theory for friendly competition remains in its infancy, because the three limited case studies (Strauss *et al.* 2015) leave us with two major questions. First, which focal host traits predict outcomes of friendly competition? Variation in disease risk might predict size of focal host epidemics, and could create an opportunity for dilution. Alternatively, variation in competitive ability might regulate density of competitor/diluters, and could constrain their impacts on disease. Either, neither, or both of these traits could predict outcomes of friendly competition. Second, which dilution mechanisms reduce disease? Either host regulation or encounter reduction might operate more strongly, and their relative importance could even depend on the metric of disease being considered. For example, if density of focal hosts becomes decoupled from infection prevalence (e.g., Civitello *et al.* 2013; Strauss *et al.* 2016), then host regulation might reduce density of infected hosts, but have no impact on infection prevalence (reminiscent of the third case study in Strauss *et al.* 2015). Strength of either or both dilution mechanisms could depend on disease risk (if only larger epidemics can be 'diluted') or competitive ability (if more numerous diluters promote each mechanism). Together, a synthetic framework linking host traits, species densities, and disease outcomes could simultaneously predict which host traits regulate friendly competition and which dilution mechanisms reduce disease.

Here, we test such a mechanistic, predictive framework, centered on variation in disease risk and competitive ability. First, we intentionally spread variation in these traits with a set of eight focal host genotypes. Then we create experimental mesocosm

epidemics for each genotype, lasting ~6-8 generations, both with and without competitor/diluters. Throughout this experiment, we track changes in density of focal hosts and competitor/diluters, infection prevalence, and density of infected hosts. Finally, we use path analysis to disentangle links among focal host traits and these mesocosm outcomes. Path models disentangle direct impacts of competitor/diluters on disease (i.e., encounter reduction) from indirect effects mediated by focal host density (i.e., host regulation; see Begon 2008), separately for both infection prevalence and density of infected hosts. Thus, we demonstrate which focal host traits predict outcomes of friendly competition, and which dilution mechanisms reduce each metric of disease. Ultimately, we aim to enhance mechanistic clarity of the dilution effect by delineating when and how diluters reduce disease in the general friendly competition module.

MATERIALS AND METHODS

Natural History of the Study System

The focal host, competitor/diluter, and parasite here all co-occur in many North American freshwater lakes. The focal host, the cladoceran *Daphnia dentifera*, frequently dominates grazer communities but often suffers autumnal epidemics caused by the virulent fungus *Metschnikowia bicuspidata* (Hall *et al.* 2010b; Strauss *et al.* 2016). Focal hosts incidentally consume infectious fungal spores while foraging (Hall et al. 2007). Infected hosts cannot recover, die from infection, and release spores after death. Competitor/diluters compete with focal hosts in many lakes (Tessier & Woodruff 2002), and by regulating density of the focal host population, can also regulate disease (Strauss *et al.* 2016). These diluters also consume fungal spores while foraging, but rarely become infected (Hall *et al.* 2009; Strauss *et al.* 2015), and hence reduce encounters

between focal hosts and parasites. Among lakes, host regulation appears to primarily reduce density of infected hosts, while encounter reduction reduces infection prevalence (Strauss *et al.* 2016). Together, these interactions exemplify friendly competition.

Trait Measurements

We quantified indices of two potentially important traits, disease risk and competitive ability, for eight different focal host genotypes (see Appendix S1 in Supporting Information for details). In short, we estimated an index of disease risk (the transmission coefficient β) by fitting a mathematical model to data from infection assays (e.g., Hall *et al.* 2007; Hall *et al.* 2012). In these assays, fifteen individuals were exposed to each of three parasite concentrations, maintained individually, and later inspected for signs of infection. The transmission coefficient was fit with maximum likelihood using the BBMLE package in R (Bolker 2008; R Development Core Team 2010). This parameter represents the probability of a focal host becoming infected, given its body length (L), density of infectious spores (Z), and the duration of spore exposure (t). We bootstrapped standard errors around means for each genotype in R.

We estimated an index of competitive ability with growth rate assays using low food resources (e.g., Hall *et al.* 2012). Mass accrual of neonates during a 5-6 day juvenile period becomes directly proportional to fitness, once adults begin investing energy in reproduction (Lampert & Trubetskova 1996). In turn, competitive ability depends on fitness when resources are limiting (reviewed in Grover 1997). Therefore, we provided hosts with low resources in our assay (0.15 mg mass/L *Ankistrodesmus falcatus* daily). We dried and weighed body mass of individuals at birth (mean N = 9.8) and other individuals 5-6 days later (mean N = 14.5). We calculated growth rate as

ln(mass accrual)/time. Finally, we bootstrapped standard errors around growth rate for each genotype in R.

Mesocosm Experiment

The mesocosm experiment crossed focal host genotype (8 levels) with presence/absence of competitor/diluters (2 levels). All combinations of treatments were replicated 4 times. Each replicate was housed in a 75-liter acid-washed polyethylene tank in a climate-controlled room and grown under a 16 L: 8 D light cycle. First, we filled tanks to 60 liters with high-hardness COMBO (artificial lake water). Then, we added initial doses of nitrogen (300 ug L^{-1} N as $NaNO_3$) and phosphorus (20 ug L^{-1} P as K_2HPO_4). We inoculated all tanks with algae (50 mg dry weight *Ankistrodesmus falcatus*) and added focal hosts (15 L^{-1}) and a single genotype of competitor/diluters (5 L^{-1}) two days later. After two weeks of growth, we began weekly sampling by mixing and sieving 1 L per tank (80 µm mesh). After the first week of sampling, we added fungal spores to all tanks (5,000 L^{-1}). Then, we continued sampling for seven additional weeks. Throughout the experiment (6-8 focal host generations), we replaced evaporated COMBO and replenished nutrients, assuming a 5% exponential daily loss rate. We tracked changes in density of focal hosts and competitor/diluters, infection prevalence, and density of infected focal hosts, using microscopes to quantify samples and diagnose infections (50X). Only 4 of 6,375 competitor/diluters examined were infected (0.06%), confirming their low disease risk. Therefore, we only focus on prevalence and density of infections in focal hosts.

Statistics

We linked trait measurements to mesocosm dynamics with univariate generalized least squares (GLS) linear models. We included an additional variance parameter (using the NLME package in R: Pinheiro & Bates 2000), if it improved model fit (likelihood ratio tests). When focal host traits served as the independent variable, we also fit a complementary linear mixed model (also using NLME) which assigned each focal host genotype a random intercept (see Appendix 3 for results). For mesocosm data, we averaged time series data for each tank over the 8 week experimental period. Our calculation of mean infection prevalence omitted days with extremely low densities of focal hosts ($< 15\ L^{-1}$), due to potentially influential sampling error.

Several sets of linear models tested specific hypotheses linking host traits to mesocosm variables. The first set tested whether our index of disease risk (i.e., the transmission coefficient, β) predicted variation in prevalence and density of infected hosts, and whether presence of competitor/diluters (denoted C) modulated these relationships. Thus, these models tested if higher disease risk created an opportunity for dilution (denoted by a $\beta \times C$ interaction). The next suite of models tested whether the index of competitive ability predicted variation in density of competitor/diluters, which subsequently mapped to focal host density, infection prevalence, and density of infected hosts. The last set evaluated whether density of focal hosts at the start of epidemics (week 2) predicted size of epidemics better than mean density of focal hosts throughout the experiment. All relationships between traits and mean densities then became the scaffolding for path models.

Path analysis tested which traits regulated friendly competition and which dilution mechanisms reduced each metric of disease (infection prevalence vs. density of infected hosts). Unfortunately, an undesirable estimation result (collinearity among parameters)

prevented fit of the most comprehensive model (linking both traits, both densities, and both metrics of disease). Given this constraint, we fit four complementary models instead. The first two determined which traits regulated friendly competition, focusing first on infection prevalence and then on density of infected hosts. The second pair of models used only one trait, but disentangled which dilution mechanism (host regulation vs. encounter reduction) reduced each metric of disease (first infection prevalence, and then density of infected hosts).

Because traits were replicated by genotype but mesocosm dynamics were replicated by tank, we specified a two-level hierarchical structure for path models with the lavvan survey package in R (Oberski 2014). These hierarchical path models were then fit using the lavaan package (Rosseel 2012), with a maximum likelihood estimator (MLM) that was robust to non-normal standard errors. We assessed model fit with a Satorra-Bentler chi-square test statistic (Satorra and Bentler 2001) and robust criteria including CFI, TLI, RMSEA, and SRMR (Hu and Bentler 1999). We extracted *P* values and standardized parameter estimates for each relationship.

RESULTS

Focal hosts varied in both traits. The transmission coefficient, β (the index of disease risk), ranged 1.8 – 5.2 $x10^{-6}$ (L $spore^{-1}$ mm^{-2}) among the 8 focal host genotypes (Fig. 1A). Hereafter, we rank our focal host genotypes by this trait (i.e., the genotype with lowest transmission rate becomes “G1”). The second trait, juvenile growth rate on low resources (the index of competitive ability), ranged 0.13 - 0.17 (day^{-1}) (Fig. 1B). Although these traits covaried positively among genotypes, the correlation was not significant (Pearson’s $P = 0.13$; Fig. 1C).

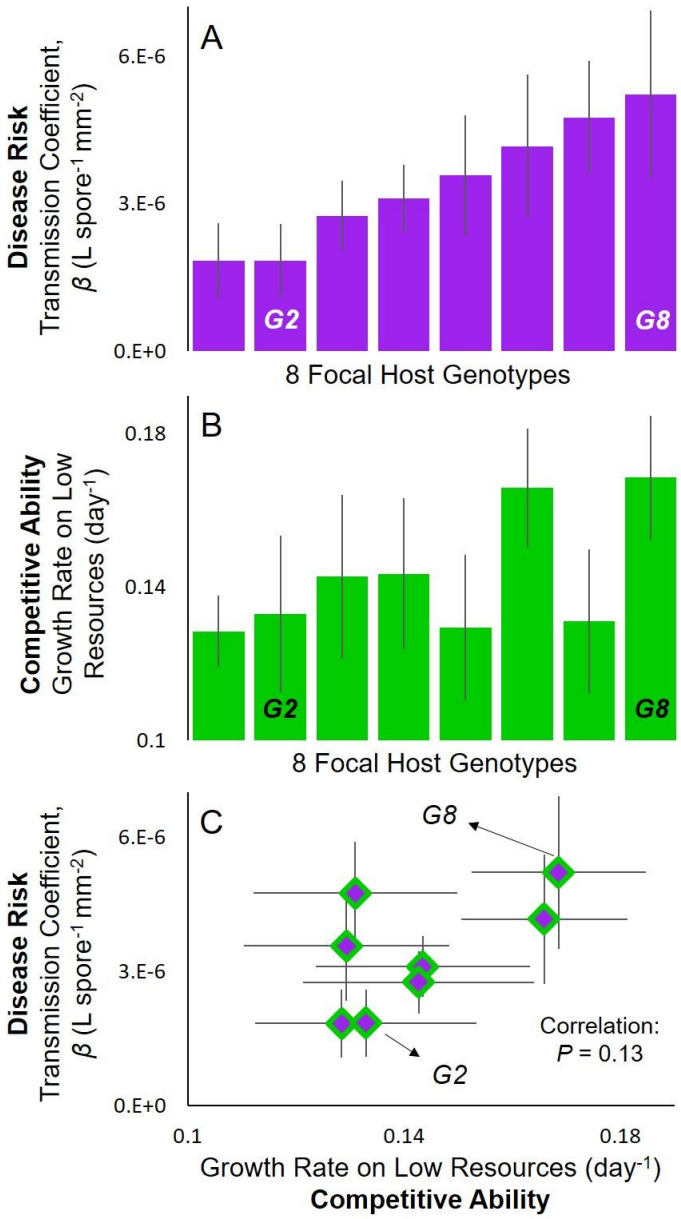

Figure 1. *Eight focal host genotypes vary in two key traits.* ***A)*** The transmission coefficient (*β*; measured with infection assays) represents an index of disease risk. **B)** Growth rate on low resources (measured with juvenile growth rate assays) represents an index of competitive ability. Focal host genotypes in A & B are both ordered according to variation in the transmission coefficient. **C)** Growth rate on low resources and the transmission coefficient (*β*) are not significantly correlated among focal host genotypes (*P* = 0.13). Two illustrative focal host genotypes (G2 & G8) are emphasized for comparison with mesocosm dynamics (see Fig. 2). Error bars are bootstrapped standard errors

Focal host genotypes also created different outcomes in the mesocosm experiment (Figs. 2, S1 & S2). Two genotypes (G2 & G8) illustrate the range of outcomes (Fig. 2). Focal host G2 featured low indices of disease risk and competitive ability (see Fig. 1). In the mesocosm experiment, G2 spread small epidemics and competed relatively weakly. Competitor/diluter density increased throughout the experiment, and competition lowered focal host density, especially during weeks 3-8

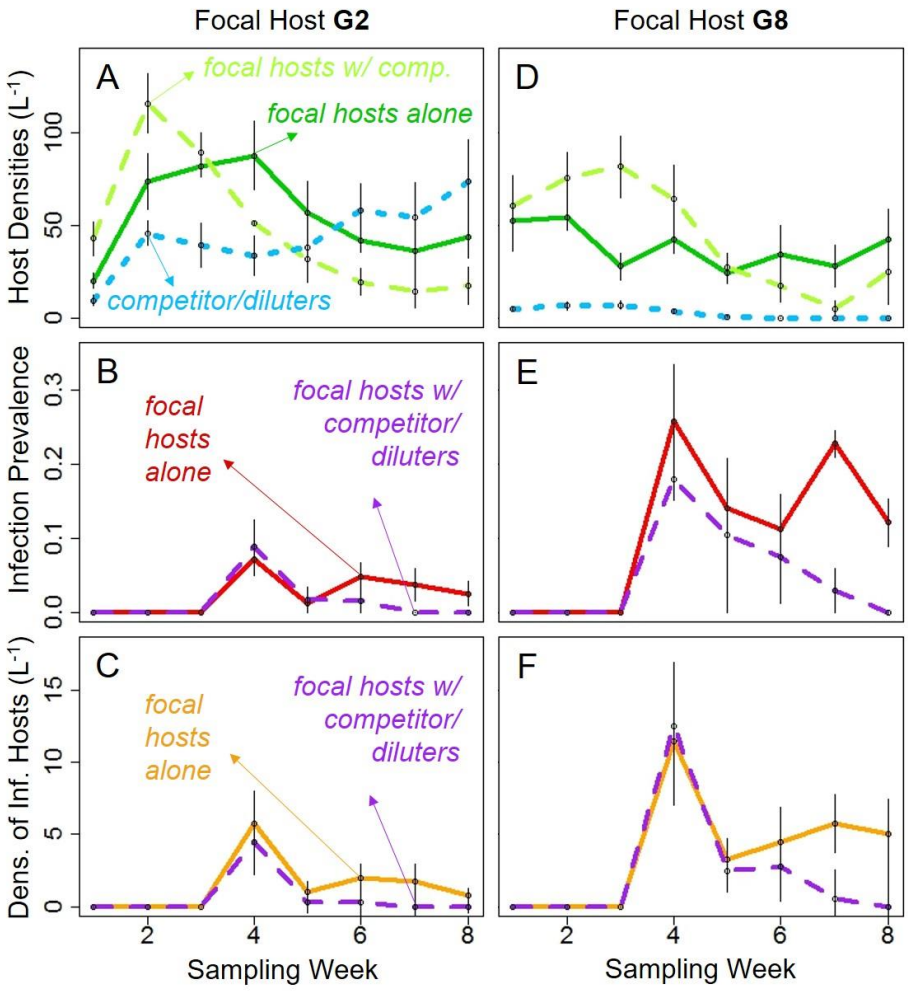

Figure 2. *Mesocosm dynamics of two illustrative focal host genotypes varying in key traits (see Fig. 1).* Time series show changes in host densities (top), infection prevalence (center), and density of infected hosts (bottom), for both focal host genotypes (columns). **A-C)** Focal host G2 features low indices of disease risk and competitive ability. A) It competes weakly and maintains low B) prevalence and C) density of infected hosts. **D-F)** In contrast, focal host G8 features high indices of disease risk and competitive ability. D) It competes strongly and maintains high E) infection prevalence and F) density of infected hosts. B, C, E & F) Competitor/diluters appear to reduce both metrics of disease, but more clearly for G8 than G2. Error bars are standard errors. Key: solid lines = focal hosts alone; dashed = focal hosts in competition; dotted = competitor/diluters in competition.

(Fig. 2A). Both prevalence and density of infected hosts remained low and were minimally reduced by competitor/diluters (Fig. 2B & C, respectively). In contrast, focal host G8 featured high indices of disease risk and competitive ability (see Fig. 1). G8

spread larger epidemics and competed relatively strongly. Competitor/diluter density remained low, and competition (on average) did not depress focal host density (Fig. 2D). Both prevalence and density of infected hosts were much higher, and were more clearly reduced by competitor/diluters (Fig. 2E & F, respectively). Mesocosm time series dynamics of the other six focal host genotypes were each qualitatively unique (see Figs. S1 & S2 in Appendix S2 of Supporting Information).

Variation in disease risk predicted prevalence and density of infected hosts in the mesocosm experiment and created an opportunity for dilution (Fig. 3). When competitor/diluters were absent, higher disease risk among focal hosts maintained higher mean infection prevalence (β effect: $P < 0.0001$; Fig. 3A). Moreover, presence of competitor/diluters (denoted *C*) reduced infection prevalence, but only for focal hosts with high disease risk (significant β x *C* interaction: $P = 0.044$, but nonsignificant *C* main effect: $P = 0.14$). Thus, variation in disease risk created an opportunity for dilution (particularly when β was high). When competitor/diluters were absent, higher disease risk also drove a higher density of infected hosts ($P = 0.0042$; Fig. 3B). However, presence of competitor/diluters did not mediate this relationship via main *C* effect or β x *C* interaction (both $P > 0.6$).

Variation in competitive ability predicted how strongly focal hosts constrained populations of competitor/diluters, which in turn impacted disease (Fig. 4). First, strongly competing focal hosts constrained competitor/diluters to lower mean densities ($P < 0.0001$; Fig. 4A). Then, higher densities of competitor/diluters constrained densities of focal hosts ($P = 0.0011$; Fig. 4B). Finally, focal host and competitor/diluter densities impacted each disease metric differently. Mean infection prevalence was reduced by higher densities of competitor/diluters ($P = 0.036$; Fig. 4C). However, it was unaffected by density of focal hosts (*Hd*), presence of competitor/diluters (*C*), or their interaction (*Hd* x *C*; all $P > 0.4$; Fig. 4D). Thus, the density of focal hosts was decoupled from infection

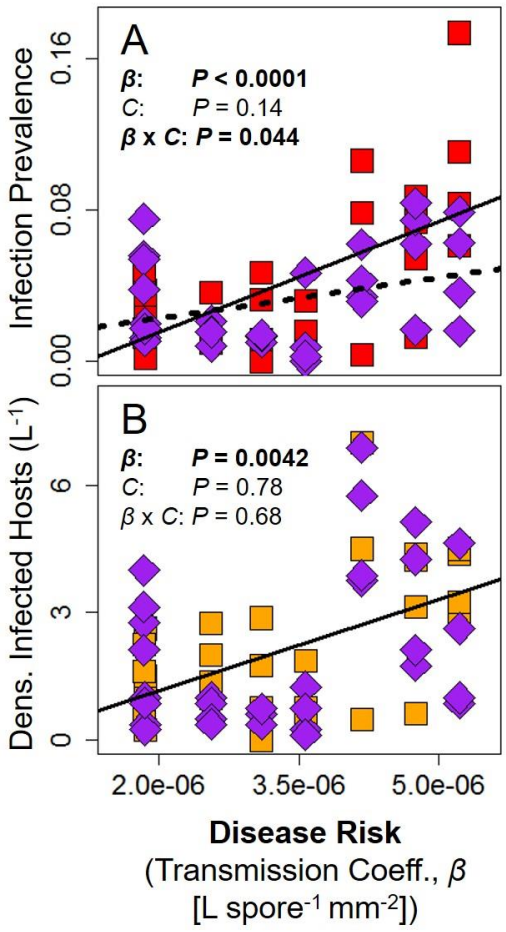

Figure 3. *Variation in disease risk (i.e., transmission coefficient,* β*) predicts epidemic size and creates an opportunity for dilution.* Infection prevalence and density of infected hosts are averaged throughout the experiment for each mesocosm tank. A) Higher disease risk in focal hosts increases mean infection prevalence. Presence of competitor/diluters reduces infection prevalence, but only for focal hosts with high disease risk. Thus, variation in disease risk creates an opportunity for dilution. B) Higher disease risk also elevates mean density of infected hosts, although presence of competitor/diluters does not mediate this relationship. *P* values come from fits of linear models. Key: *β* = disease risk; *C* = presence of competitor/diluters; *β* x *C* = interaction; red squares = infection prevalence alone; orange squares = density of infected hosts alone; purple diamonds = with competitor/diluters; solid lines = significant *β* effect; dashed line = significant *β* x *C* interaction.

prevalence. Mean density of infected hosts also appeared to be reduced by higher densities of competitor/diluters ($P = 0.0004$; Fig. 4E). In contrast with infection prevalence, density of infected hosts was elevated by higher densities of focal hosts (*Hd* effect: $P = 0.014$; Fig. 4F), although presence of competitor/diluters neither contributed to this model as main effect (*C)* or interaction (*Hd* x *C*; both $P > 0.5$).

Complementary analyses using density of focal hosts from week 2 only (when spores were added) mirrored these results (see Appendix S3). Density of focal hosts in week 2 correlated strongly with mean focal host density (*D2* effect: $P < 0.0001$; Fig.

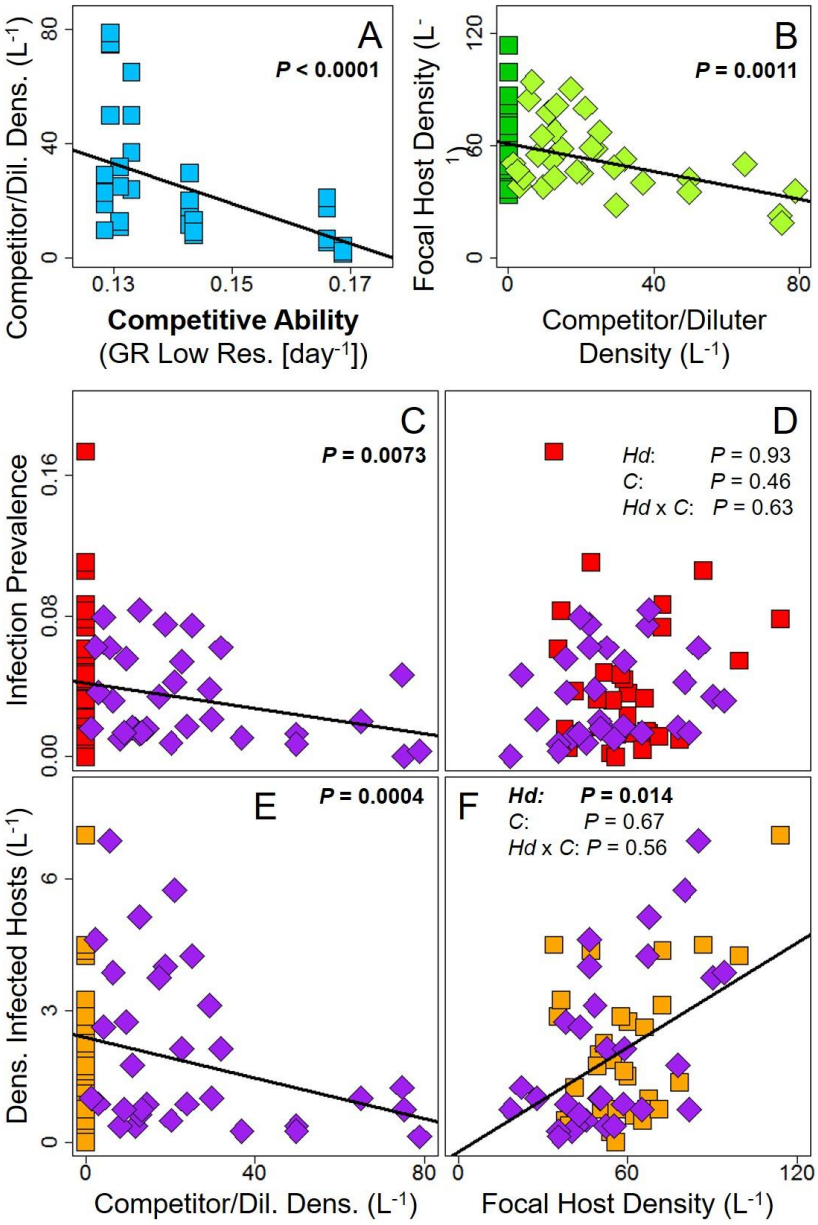

Figure 4. *Variation in competitive ability (i.e., juvenile growth rates on low resources) predicts competition outcomes and leads to impacts on disease.* A) Genotypes of focal hosts with higher competitive ability better constrain competitor/diluters. B) In turn, higher densities of competitor/diluters constrain focal hosts. Infection prevalence is C) lowered by high densities of competitor/diluters, but D) unaffected by focal host density. In contrast, density of infected hosts is both E) lowered by high densities of competitor/diluters and F) elevated by high densities of focal hosts. *P* values come from fits of linear models. Key: *C* = presence of competitor/diluters; *Hd* = density of focal hosts; *Hd* x *C* = their interaction; dark green squares = focal hosts alone; light green diamonds = with competitor/diluters; red squares = infection prevalence alone; orange squares = density of infected hosts alone; purple diamonds = with competitor/diluters.

S3A), but it did not impact mean infection prevalence (*D2* effect: $P = 0.27$; Fig. S3B), and it elevated mean density of infected hosts (*D2* effect: $P = 0.048$; Fig. S3C). Thus, density of focal hosts and infection prevalence were robustly decoupled. Consequently, host regulation could not impact infection prevalence, but may have still reduced density of infected hosts.

All four path models fit well (see Table S2 in Appendix S3 for diagnostic statistics; see Tables S3-S6 for parameter estimates). The first pair of path models determined which host traits regulated friendly competition (Fig. 5). Both disease risk and competitive ability simultaneously regulated both infection prevalence (Fig. 5 A) and density of infected hosts (Fig. 5 B). Paths in both models were qualitatively or even quantitatively similar. Higher disease risk raised both metrics of disease (both $P < 0.01$), and higher competitive ability reduced density of competitor/diluters (both $P = 0.018$). In turn, higher densities of competitor/diluters reduced both infection prevalence ($P = 0.015$; Fig. 5 A) and density of infected hosts ($P = 0.004$; Fig. 5 B). The two traits covaried in both models, although not significantly (both $P = 0.15$). In other words, focal hosts with lower disease risk benefited from less baseline disease. Simultaneously, weaker competitors benefited relatively more from disease dilution, since competitor/diluters were more dense.

The second pair of path models determined which dilution mechanism reduced each metric of disease (Fig. 6). Although competitor/diluters exerted superficially similar impacts on each disease metric (Fig. 5 A & B), these effects arose through fundamentally different mechanisms. Specifically, competitor/diluters reduced infection prevalence via encounter reduction (Fig. 6 A), but reduced density of infected hosts via host regulation (Fig. 6 B). To understand this difference, first consider similarities between the two models: higher disease risk increased each metric of disease (both $P < 0.01$), and higher densities of competitor/diluters constrained densities of focal hosts in

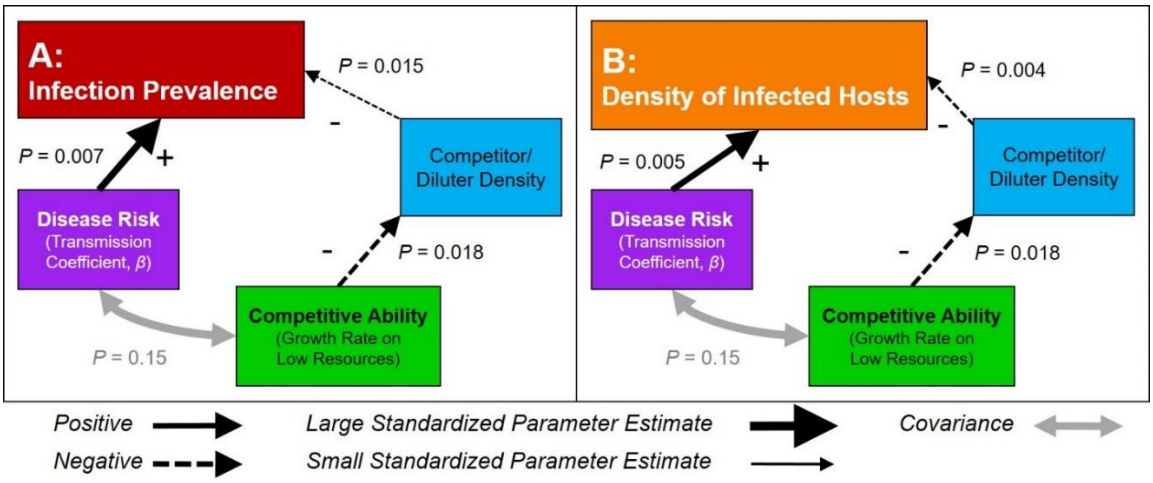

Figure 5. *Path models uncover which focal host traits regulate friendly competition.* All mesocosm variables are averaged over time for each tank. Both variation in disease risk and competitive ability simultaneously regulate A) infection prevalence and B) density of infected hosts. Paths regulating each disease metric appear qualitatively similar. The two traits covary, but nonsignificantly. Higher disease risk always increases disease. Simultaneously, lower competitive ability always increases density of competitor/diluters. In turn, higher density of competitor/diluters always appear to reduce disease. Thus, these impacts of competitor/diluters on A) infection prevalence vs. B) density of infected hosts appear superficially similar (but see Fig. 6). Solid arrows represent positive coefficients; dashed arrows represent negative coefficients; arrow weights are standardized effect sizes.

both cases (both $P = 0.001$). However, differences between the models stemmed from the relationship between focal host density and each metric of disease. Focal host density was strongly correlated with density of infected hosts ($P = 0.026$ Fig. 6B). This strong link allowed host regulation (competition with diluters) to indirectly reduce density of infected hosts. In other words, density of infected hosts depended on density of focal hosts, which depended on density of competitor/diluters. Once this path model accounting for host regulation, the direct link between competitor/diluter density and density of infected hosts (signaling encounter reduction) became nonsignificant

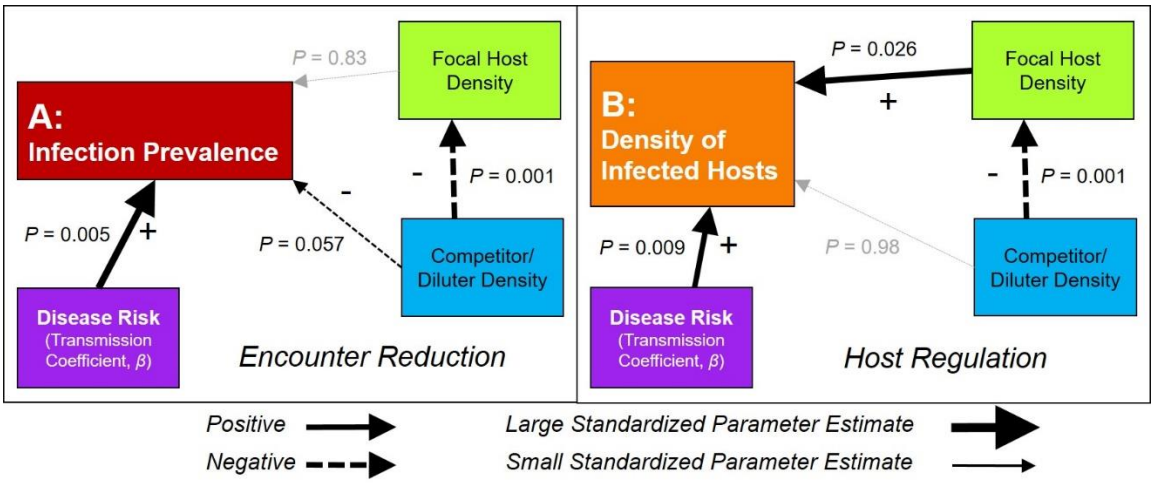

Figure 6. *Path models uncover which dilution mechanisms reduce each metric of disease.* A) Encounter reduction lowers infection prevalence, but B) host regulation lowers density of infected hosts. All mesocosm variables are averaged over time for each tank. In both path models, higher density of competitor/diluters lowers density of focal hosts. However, A) infection prevalence is decoupled from focal host density. Therefore, host regulation is not supported. Instead, high densities of competitor/diluters reduce infection prevalence directly, likely via **encounter reduction**. In contrast, B) focal host density correlates strongly with density of infected hosts. Competitor/diluters indirectly regulate density of infections via their competitive impacts on focal host density (i.e., **host regulation**). This indirect impact on disease outweighs competitor/diluters' direct effect. In other words, encounter reduction is weak but host regulation is strong. Solid arrows represent positive coefficients; dashed arrows represent negative coefficients; arrow weights are standardized effect sizes.

(*P* = 0.98). Thus, the bivariate relationship between density of competitor/diluters and density of infected hosts (Fig. 4E) was merely a correlational shadow. In contrast, focal host density became decoupled from infection prevalence (*P* = 0.83; Fig 6A). In turn, despite competition with diluters, these density-mediated effects could not impact infection prevalence. Instead, a much stronger direct link between density of competitor/diluters and infection prevalence (P = 0.057) signaled encounter reduction (i.e., consumption of free-living parasites).

DISCUSSION

Friendly competition represents a mechanistic frontier for dilution effect theory by combining two general dilution mechanisms: encounter reduction and host regulation (Keesing *et al.* 2006; Johnson *et al.* 2015). This simple community module of focal hosts, parasites, and competitor/diluters nevertheless produces a variety of outcomes for focal host density, infection prevalence, and density of infected hosts (Cáceres *et al.* 2014; Strauss *et al.* 2015). A fully mechanistic framework for friendly competition could delineate which focal host traits regulate these outcomes and which dilution mechanisms reduce each metric of disease. Here, we empirically tested this framework. First, we selected eight focal host genotypes to spread a range of competitive ability and disease risk (Fig. 1). Then, we tested outcomes of friendly competition for each genotype in a mesocosm experiment lasting ~6-8 focal host generations. Both traits regulated disease together (Fig. 5): higher disease risk increased the size of epidemics and created an opportunity for dilution (Fig. 3). Simultaneously, lower competitive ability elevated densities of competitor/diluters (Fig. 4A) and facilitated their reduction of disease (Fig. 4C&E). However, competitor/diluters reduced each metric of disease through a different mechanism: encounter reduction reduced infection prevalence (Fig. 6 A), while host regulation reduced the density of infected hosts (Fig. 6 B). Our experiment lays ecological foundations of a mechanistic framework for friendly competition, and enhances predictive clarity for the dilution effect in a variety of disease systems.

Variation in disease risk determined size of focal host epidemics and created an opportunity for dilution. Each metric of disease was impacted slightly differently. First, higher disease risk elevated mean infection prevalence when competitor/diluters were absent (Fig. 3A). In turn, their presence reduced infection prevalence, but only for focal hosts with high disease risk (i.e., the β x C interaction; Fig. 3A). In other words, as disease risk declined, competitor/diluters became irrelevant for transmission, and the

dilution effect reduced infection prevalence less. Thus, although higher disease risk may increase infection prevalence (potentially harming focal host fitness when parasites are virulent), it could also create opportunities for dilution. Focal host populations might maintain variation in this potentially costly trait when tradeoffs link high disease risk with faster pace of life (Johnson *et al.* 2012) or superior resource acquisition (Hall *et al.* 2010a). In turn, dilution effects may be easier to detect under these conditions (e.g., Johnson *et al.* 2013; Strauss *et al.* 2016, respectively). The generality of similar tradeoffs could predict how frequently focal hosts maintain high disease risk, and hence create opportunities for dilution (Ostfeld & Keesing 2012). In our experiment, higher disease risk also elevated the density of infected hosts (Fig. 3B). This second disease metric did not respond to mere presence of competitor/diluters (Fig. 3B), but did decline with their density (Fig. 4E).

In turn, density of competitor/diluters, and hence disease outcomes, were impacted by competitive ability of focal hosts. Specifically, lower competitive ability elevated the density of competitor/diluters (Fig. 4A). Higher density of competitor/diluters, in turn, constrained density of focal hosts (Fig. 4B) and appeared to decrease both infection prevalence (Fig. 4C) and the density of infected hosts (Fig. 4E). In other words, variation in competitive ability regulated outcomes of the dilution effect by shifting the relative densities of competitor/diluters and focal hosts. This balance between densities of focal hosts and competitor/diluters also imposed a fitness constraint for focal hosts. Weakly competing focal hosts benefited more from disease reduction, but risked being outcompeted and possibly even competitively excluded. In contrast, stronger competitors minimized costs of competition, but benefited less from disease dilution. Thus, all else equal, focal hosts competing with competitor/diluters during epidemics cannot simultaneously maximize benefits of competitive superiority and disease reduction.

Yet all else may not be equal, if competitive ability and disease risk covary. Both focal host traits simultaneously regulated outcomes of friendly competition here (Fig. 5). In turn, this co-regulation demands a better understanding of the covariation between these traits (e.g., Kraaijeveld & Godfray 1997; Duncan *et al.* 2011). Disease risk and competitive ability covaried weakly among the eight genotypes here (Fig. 1C). High disease risk also correlates with superior resource acquisition in some populations of this plankton focal host (Auld *et al.* 2013). Thus, when focal hosts are superior competitors, they may suffer higher disease risk. Higher disease risk magnifies the impacts of competitor/diluters on infection prevalence (Fig. 3A). Therefore, diluters might still reduce disease for these focal hosts, despite being relatively rare (e.g., focal host G8; Fig. 2). On the other hand, extremely high disease risk and competitive ability could catalyze "dilution failure," where large uncontrollable epidemics spill over into the sparse diluter population (Strauss *et al.* 2015). Thus, variation and covariation among genotypes shapes the trait combinations that together drive outcomes of friendly competition. Variation and covariation among genotypes (see Day & Gandon 2007) also likely shapes traits of rapidly evolving host populations (e.g., Duffy *et al.* 2012). In turn, our mechanistic framework could link evolving competitive ability and disease risk to eco-evolutionary outcomes of friendly competition (see Strauss et al. in prep.). Variation and covariation in host traits remain an understudied key to this eco-evolutionary frontier of dilution effect research.

Different dilution mechanisms reduced each metric of disease, partly because infection prevalence became decoupled from focal host density (Figs. 4D & S3B). Host density was strongly correlated with density of infected hosts (Figs. 4F & S3C), which allowed host regulation (competition with diluters) to reduce this metric of disease (Fig. 6B). In this path model, the direct link between density of competitor/diluters and infected hosts (signaling encounter reduction) became nonsignificant, even though this

relationship appeared significant when tested univariately (Fig. 4E). Instead, this apparent direct impact of competitor/diluters was merely a correlational shadow of their indirect effects, mediated by focal host density (see Begon 2008). In contrast, focal host density was directly decoupled from infection prevalence, so impacts on focal host density did not alter prevalence. Instead, encounter reduction reduced infection prevalence directly (Fig. 6A). Prevalence may been become decoupled from host density due to host interference (Civitello *et al.* 2013) or other mechanisms (Fenton *et al.* 2002). Regardless, these results echo our field patterns: focal host density correlates with density of infected hosts but not infection prevalence. In turn, competitor/diluters reduce infection prevalence directly via encounter reduction, but reduce density of infected hosts indirectly via host regulation (Strauss *et al.* 2016). Could infection prevalence generally be more sensitive to encounter reduction, while density of infected hosts depends more on host regulation? At the very least, understanding when density becomes decoupled from infection prevalence (perhaps especially for vector-borne diseases) may help predict when host regulation does and does not matter for the dilution effect (see Randolph & Dobson 2012).

More generally, mechanistic insight into each dilution mechanism and disease metric helps clarify the dilution effect from a variety of perspectives. First, infection prevalence can represent the cost of virulent infection from the focal host's perspective. Thus, if a dilution effect lowers infection prevalence, it might boost focal host fitness. This perspective seems especially appealing when focal hosts are crops (Boudreau 2013), livestock (Huang *et al.* 2013), or declining native species (Thieltges *et al.* 2009) or charismatic taxa (Johnson *et al.* 2013; Venesky *et al.* 2014). Diluters that reduce disease via encounter reduction may especially benefit these focal hosts. In contrast, density of infected hosts can represent disease risk to humans in zoonotic diseases like Lyme disease (Ogden & Tsao 2009), hantavirus (Suzan *et al.* 2009), and

schistosomiasis (Johnson *et al.* 2009). In these cases, diluters that reduce disease via host regulation (i.e., competitors) may more effectively reduce density of infected hosts and hence minimize disease risk to humans. While encounter reduction and host regulation operate together in friendly competition, these insights may also apply to disease systems featuring only one dilution mechanism. In these cases, pinpointing the relevant dilution mechanism could help predict how diluters reduce either metric of disease.

Our mechanistic framework for friendly competition could be readily expanded. First, it could incorporate variation in traits of competitor/diluters. Variation in their disease risk, competitive ability, and encounter rates with parasites could clarify what types of diluters have what impacts on disease. Second, expansions could also include traits of parasites, especially when infection and competition depend on matches between focal host and parasite genotypes (e.g., Refardt & Ebert 2012). In genetically diverse focal host populations, resistant focal host genotypes could even serve as diluters, creating an opportunity for intraspecific friendly competition. Third, a predator could be added (see Grover 1997). Predation can determine frequencies of focal hosts and diluters (Strauss *et al.* 2016) and could flip the hierarchy of competitive abilities (Hall *et al.* 2012). If the positive covariance between competitive ability and disease risk becomes negative, predators could create an entirely new suite of trait-dependent outcomes for friendly competition (e.g., high disease risk and weak competitive ability). Finally, an eco-evolutionary framework (Strauss et al. in prep.) could better predict outcomes of friendly competition in nature, when focal hosts exist in genetically diverse (rather than isoclonal) populations. Eco-evolutionary outcomes would likely depend on variation and covariation in host traits (Day & Gandon 2007) and selection imposed by competitors and parasites (Duffy *et al.* 2012). Each of these expansions could be

layered into our framework for friendly competition with mathematical models, and at least in our study system, accompanied and evaluated with experiments.

The friendly competition framework strengthens theory for local dilution mechanisms. Future synthesis must expand this niche-focused, trait-based approach to the broader metacommunity scale where the dilution effect emerges (see Joseph *et al.* 2013; Mihaljevic *et al.* 2014). Here, at the local scale, the dilution effect was strongest when focal hosts were outcompeted and became numerically rare relative to diluters (Fig. 5). Yet at the metacommunity scale, the dilution effect pattern (high host diversity leads to lower disease) requires that focal hosts are common among sites while diluters are relatively rare. Then, as diversity declines, diluters are lost from communities and disease increases (Ostfeld & Keesing 2000, 2012). At first glance, these local vs. regional perspectives may seem contradictory. Yet this tension could be resolved by better understanding the persistence and abundance of diluters among sites. Local coexistence between focal host and diluters might depend on niche space created by parasites and some traits (i.e., competitive ability and disease risk). On the other hand, other traits (e.g., dispersal ability) or patch heterogeneity (e.g., variation in predation among sites (Strauss *et al.* 2016)) could become more important at the metacommunity scale. Moving forward, theory for the dilution effect must merge these spatial scales, and synthesize the mechanistic insights gained from each perspective.

The dilution effect remains controversial, in part because local interactions among focal hosts, parasites, and diluters have been understudied. The friendly competition model offers a great opportunity to develop theory for these local interactions because it combines encounter reduction and host regulation to produce a variety of predictable outcomes. Here, we empirically evaluated a mechanistic trait-based framework for friendly competition, ultimately aiming to develop predictive theory for the dilution effect. With path analysis, we uncovered which focal host traits regulated

outcomes of friendly competition, and which dilution mechanisms reduced each metric of disease (prevalence vs. density of infected hosts). Focal hosts benefited most from disease dilution when they suffered high disease risk or competed weakly. However, infection prevalence was primarily reduced by encounter reduction, a direct effect of competitor/diluters, while the density of infected hosts was more strongly reduced by host regulation, an indirect effect of competitor/diluters. This difference stemmed in part from the decoupling of infection prevalence from focal host density. Our modular, mechanistic framework for friendly competition could readily incorporate traits of diluters or parasites, predators, or eco-evolutionary feedbacks. Future theory needs to expand this perspective to the metacommunity scale. Thus, friendly competition represents a mechanistic, modular platform for dilution effect theory.

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CHAPTER 3 LITERATURE CITED

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CHAPTER 3 SUPPORTING INFORMATION

Appendix S1

In this appendix, we provide additional methodological details for our trait measurement assays. We quantified indices of two potentially important traits, 'disease risk' and 'competitive ability', for eight different focal host genotypes. All genotypes were chosen from existing laboratory cultures that had been isolated from lakes in southwestern Michigan or southwestern Indiana. Using limited prior knowledge of these genotypes, we selected focal host genotypes with the aim of spreading a range of both traits. Prior to trait measurement assays, all genotypes were grown in isoclonal cultures and fed high densities of high quality laboratory-cultured algae (2.0 mg mass/L *Ankistrodesmus falcatus*). Cultures were maintained in high hardness COMBO (artificial lake water media) under ideal conditions for three generations, in order to standardize any maternal affects.

Disease Risk: We calculated an index of disease risk (the transmission coefficient, β) from infection assays. This transmission coefficient represents the probability of a focal host becoming infected, given density of infectious spores (Z), the duration of spore exposure (t), and body length of the focal host (L). Disease transmission depends on body length, because larger hosts encounter parasites at a higher foraging rate (Hall *et al.* 2007). For the assay, we first reared cohorts of neonates of each isoclonal line (fed 1.0 mg mass/L/day of highly edible algal food, *Ankistrodesmus*). After 5 days, individuals were isolated in 15 mL of media. Fifteen of these individuals were exposed to each of three densities of fungal spores (Z): 75, 200, or 393 spores/mL (at 1.0 mg mas/L/day of algal food). Spores (< 6 weeks old) were all reared in a standard focal host genotype. After ~8 hours of exposure (t), we measured

body length of all individuals (*L*) with a dissecting microscope and micrometer. Thereafter, we transferred each individual to a fresh 50 mL tube of media daily, until death. Dead individuals were visually inspected with the dissecting microscope in order to diagnose infection. Individuals that died too early to determine infection were omitted from the analysis. This assay was conducted in three different experimental blocks, with 2 isoclonal lines repeated among blocks, in order to control for any block effects (due to potential variation in spore infectivity).

To estimate the transmission coefficient (β) from this transmission assay, we simplified a previous mathematical model (e.g., Hall *et al.* 2007; Hall *et al.* 2012). This model assumes that initial density of susceptible hosts in the assay (S_i; one per tube) decreases as susceptible hosts (S) contact spores (Z) at rate βL^2, where β is a size-controlled transmission coefficient, and L^2 is proportional to surface area. Specifically, $\frac{dS}{dt} = -\beta L^2 SZ$. Solving this equation for the final density of susceptible hosts (S_f), after exposure time (t), yields: $S_f = S_i \exp(-\beta L^2 Z t)$. We estimated the transmission coefficient (β) for each isoclonal line, using maximum likelihood and the BBLME package in R (Bolker 2008; R Development Core Team 2010). The binomial distribution (infected or not) served as the likelihood function. After controlling for block effects, we bootstrapped standard errors for each focal host genotype.

Competitive Ability: We calculated an index of competitive ability with juvenile growth rate assays on low resources (e.g., Hall *et al.* 2012). Mass accrual of neonates during a 5-6 day juvenile period becomes directly proportional to fitness, once adults begin investing energy in reproduction (Lampert & Trubetskova 1996). In turn, competitive ability depends on fitness when resources are limiting (reviewed: Grover 1997). Thus, focal hosts with high juvenile growth rates on low food resources should become strong competitors.

To calculate juvenile growth rate, we first isolated cohorts of neonates (< 24 hours old) for each focal host genotype. We obtained initial day 0 mass measurements (m_i), by drying and weighing 6-13 neonates (mean N = 11.1 per genotype) with a Mettler microbalance (Mettler-Toledo, Columbus, Ohio, USA). We also placed 11-18 live neonates (mean N = 14.5 per genotype) in separate 50 mL tubes of media. Each day, we transferred these individuals into fresh media (fed 0.15 mg mass/L *Ankistrodesmus* daily). Then, after 5 or 6 days (*d*), we dried and weighed these individuals, yielding final mass estimates (m_f). With these data, we calculated juvenile growth rate on low resources (*GR*) as the mean for each combination of initial and final mass estimates: $GR = [\ln(m_f) - \ln(m_i)] \,/\, d$. Finally, we bootstrapped standard errors around means for each focal host genotype in R.

Appendix S2

In this appendix, we display mesocosm time series for each additional focal host genotype: G1, G3, and G4 (Fig. S1), and G5, G6, and G7 (Fig. S2).

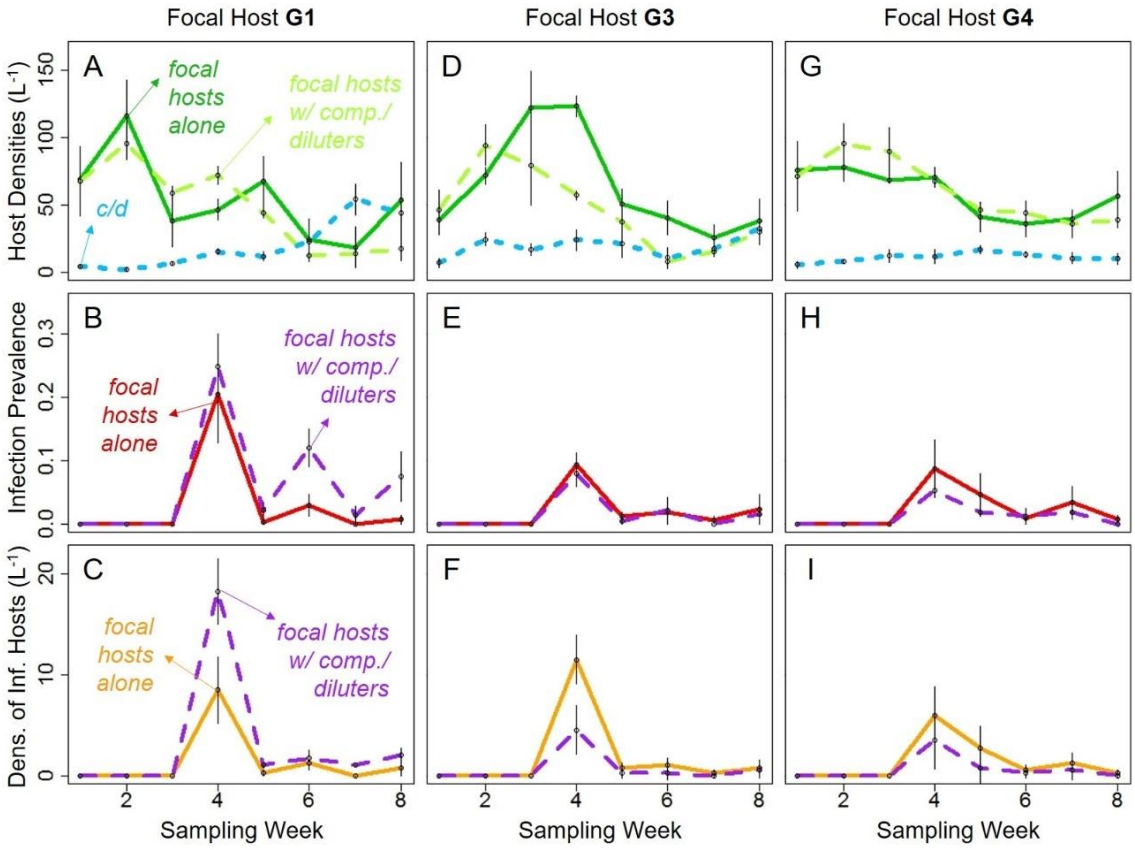

Figure S1. Mesocosm dynamics of three focal host genotypes varying in key traits (see Fig. 1). Time series show changes in host densities (top), infection prevalence (center), and density of infected hosts (bottom), for each focal host genotype (columns). Focal host G1 (left column) A) competed weakly and maintained moderate B) infection prevalence and C) density of infected hosts. In contrast, focal host G3 (center column) D) competed moderately, and maintained low E) infection prevalence and C) density of infected hosts. Finally, focal host G4 (right column) G) competed strongly, but also maintained low H) infection prevalence and I) density of infected hosts. Among focal hosts, competitor/diluters had various impacts on both metrics of disease (see Figs. 3-6 for quantitative synthesis). Error bars are standard errors. Solid lines = focal hosts alone; dashed = focal hosts in competition; dotted = competitor/diluters in competition.

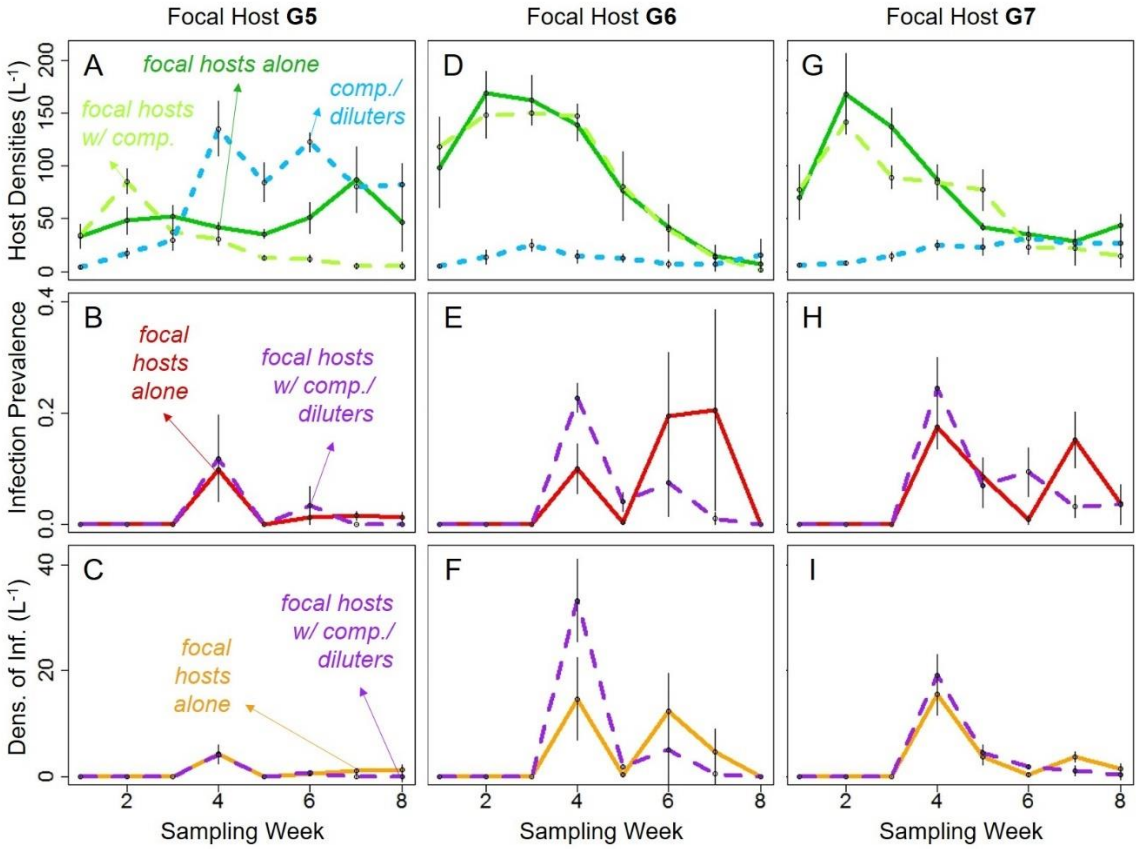

Figure S2. Mesocosm dynamics of three focal host genotypes varying in key traits (see Fig. 1). Time series show changes in host densities (top), infection prevalence (center), and density of infected hosts (bottom), for each focal host genotype (columns). Focal host G5 (left column) A) competed weakly and maintained low B) infection prevalence and C) density of infected hosts. In contrast, focal host G6 (center column) D) competed moderately, and maintained moderate E) infection prevalence and C) density of infected hosts. Finally, focal host G7 (right column) G) competed strongly and also maintained moderate H) infection prevalence and I) density of infected hosts. Among focal hosts, competitor/diluters had various impacts on both metrics of disease (see Figs. 3-6 for quantitative synthesis). Error bars are standard errors. Solid lines = focal hosts alone; dashed = focal hosts in competition; dotted = competitor/diluters in competition.

Appendix S3

In this appendix, we provide two additional analyses. First, for each link between focal host traits and a mesocosm variable, we compare results of simple linear models with results of more complex linear mixed models. We summarize the differences in Table S1. Second, we test whether epidemic size correlates with focal host density during week 2 (when spores were added), instead of mean focal host density throughout the experiment. We depict these results in Figure S3.

Simple linear models vs. linear mixed models:

Our goal is to predict outcomes of friendly competition from variation in focal host traits. Therefore, we manipulated focal host traits in our experiment, and traits serve as the independent variable in several of our analyses (Figs. 3 & 4A). However, measurement error likely impacted these trait measurements among genotypes. Hence, a more robust statistical approach could also include focal host genotype as a random effect in these models. Incorporating this mixed model structure (random intercept only) tended to raise *P* values relative to *P* values in the corresponding simpler linear models. Five of seven significant relationships remained significant, and the remaining two became trends (Table S1). However, the added complexity in these univariate models does not alter our hierarchical path models. Indeed, the primary purpose of the linear models is to help visualize the relationships underlying the path models, not merely to assign statistical significance to these isolated bivariate relationships. Thus, while the more complex mixed models weaken two of our univariate statistical results, they do not qualitatively change any of our final conclusions.

Table S1. Comparisons between simple linear models and more complex linear mixed models: linkages between focal host traits and mesocosm outcomes.

Focal Host Trait	Covariate	Independent Variable	Figure Panel	Linear Model Results	Mixed Model Results
Disease Risk (β)	Pres./Abs. of Competitor/ Diluters (C)	Infection Prevalence	1A	**β: $P < 0.0001$** C: $P = 0.14$ **β x C: $P = 0.044$**	**β: $P = 0.017$** C: $P = 0.087$ **β x C: $P = 0.021$**
Disease Risk (β)	Pres./Abs. of Competitor/ Diluters (C)	Density of Infected Hosts	1B	**β: $P = 0.0042$** C: $P = 0.78$ β x C: $P = 0.68$	β: $P = 0.096$ C: $P = 0.69$ β x C: $P = 0.53$
Competitive Ability	None	Density of Comp./Dil.	4A	**$P < 0.0001$**	$P = 0.097$

Focal host density during week 2:

In our primary analyses, mean focal host density elevated mean density of infected hosts ($P = 0.014$; Fig. 4F), but did not impact mean infection prevalence ($P = 0.93$; Fig. 4D). This decoupling between host density and infection prevalence may seem surprising. In order to evaluate the robustness of this result, we tested whether focal host density during week 2 (when fungal spores were added) might impact mean infection prevalence more clearly. However, week 2 focal host density was strongly correlated with mean focal host density ($P < 0.0001$; Fig. S3A). In this model, presence of competitor/diluters also lowered mean focal host density ($P = 0.037$), but did not significantly interact with week 2 density ($P = 0.19$). Because week 2 density and mean density were highly correlated, the impacts of each density metric on disease were qualitatively similar. Mean infection prevalence was still not impacted by week 2 density

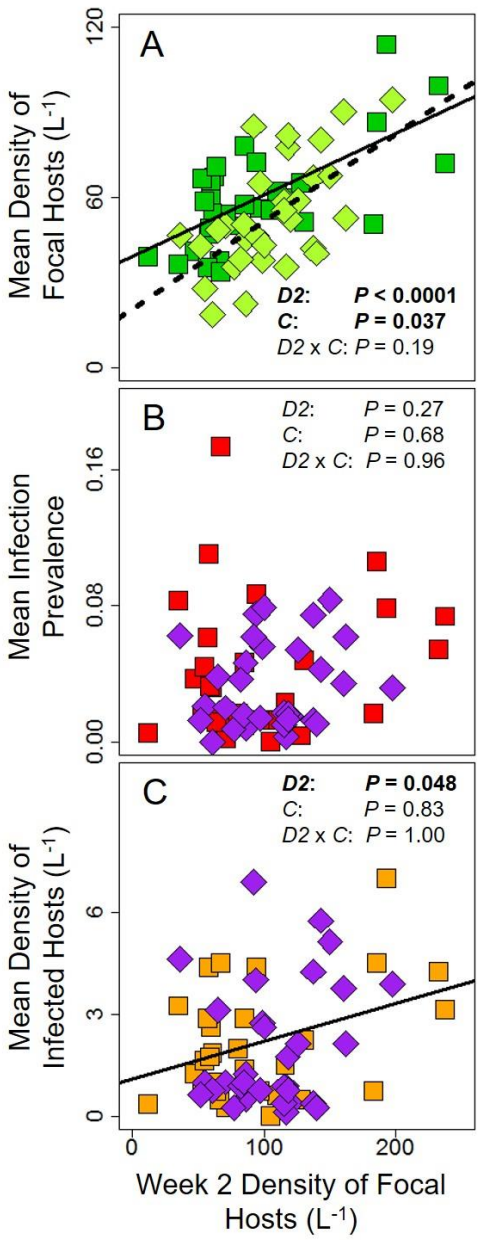

Figure S3. *Density of focal hosts during week 2 (when spores were added) correlates with mean focal host density and impacts disease metrics accordingly.* A) Higher focal host density during week 2 correlates with higher mean focal host density throughout the experiment. Hence, impacts of week 2 density on disease mirror impacts of mean focal host density. Specifically, B) it does not impact mean infection prevalence, but C) elevates mean density of infected hosts. *P* values are results of linear models. Key: *D2* = focal host density during week 2; *C* = presence of competitor/diluters; *D2* x *C* = their interaction; dark green squares = focal hosts alone; light green diamonds = with competitor/diluters; red squares = infection prevalence alone; orange squares = density of infected hosts alone; purple diamonds = with competitor/diluters; solid lines = significant *D2* effect; dashed line = significant *D2* x *C* interaction.

of focal hosts, presence of competitor/diluters, or their interaction (all $P > 0.2$; Fig. S3B). Finally, higher week 2 densities of focal hosts still elevated mean densities of infected hosts ($P = 0.048$; Fig. S3C). Presence of competitor/diluters was not significant, either as main effect or interaction (both $P > 0.8$). Thus, the decoupling of host density and infection prevalence is a robust result.

Appendix S4

In this appendix, we provide additional details of our path models. First we summarize the test statistics used to judge each model (Table S2). Then, we report parameters of models featured in Fig. 5A (Table S3), Fig. 5B (Table S4), Fig. 6A (Table S5), and Fig. 6B (Table S6).

Table S2. Test statistics, cutoff criteria for determining good model fit, and statistics of all four path models (see Fig. 5). Test statistics exceeding the desired cutoff criteria confirm that the hypothesized model is a relatively good fit for the observed data (Hu and Bentler 1999). All results use robust Satorra-Bentler chi square (Satorra and Bentler 2001).

Test Statistic	**Desired Cutoff**	**Fig. 5A**	**Fig. 5B**	**Fig. 6A**	**Fig. 6B**
Satorra-Bentler	*P* value	*P* = 0.787	*P* = 0.621	*P* = 0.734	*P* = 0.734
Chi Square	> 0.05	[1]*df* = 2	*df* = 2	*df = 1*	*df* = 1
Comparative Fit Index (CFI)	CFI > 0.95	1.000	1.000	1.000	1.000
Tucker Lewis Index (TLI)	TLI > 0.95	1.799	1.569	2.222	1.843
Root Mean Square Error of Approx. (RMSEA)	RMSEA < 0.06	0.000 [2](0.000: 0.122)	0.000 (0.000: 0.101)	0.000 (0.000: 0.000)	0.000 (0.000: 0.000)
Stand. Root Mean Square Residual (SRMR)	SRMR < 0.08	0.019	0.038	0.027	0.030

Key to abbreviations: [1]*df* = degrees of freedom; [2] 90% confidence interval

Table S3. Parameters for the path model in Fig. 5A. Bold lines indicate significant or trending relationships.

Dep. Var. [1] / Model Component	Explanatory Variable	Par.[1] Est.	SE[1]	Z-value (Wald Statistic)	*P* Value	Stand. Par. Est.[1]
Infection Prevalence ~	**Transmission Coefficient, *β***	**0.126**	**0.046**	**2.705**	**0.007**	**0.458**
	Competitor/Diluter Density	**-0.024**	**0.010**	**-2.442**	**0.015**	**-0.148**
Comp./Diluter Density ~	**Growth Rate Low Resources**	**-0.415**	**0.176**	**-2.357**	**0.018**	**-0.309**
Modeled Covariances:	Transmission Coefficient, *β* ~~ Growth Rate Low Res.	103.7	72.44	1.432	0.152	0.580
Intercepts:	Infection Prevalence	-0.197	1.673	-0.118	0.906	-0.060
	Competitor/Diluter Density	71.69	25.25	2.839	0.005	3.583
	Transmission Coeff., *β*	33.70	4.587	7.347	0.000	2.812
	Growth Rate Low Res.	142.4	5.634	25.282	0.000	9.540
Variances:	Infection Prevalence	8.029	2.443			0.744
	Competitor/Diluter Density	362.0	191.1			0.904
	Transmission Coefficient, *β*	143.6	45.38			1.000
	Growth Rate Low Resources	222.9	90.49			1.000

[1] Key to abbreviations: Dep. Var. = dependent variable; Par. Est. = parameter estimate; SE: = Standard error; Stand. = standardized

Table S4. Parameters for the path model in Fig. 5B. Bold lines indicate significant or trending relationships.

Dep. Var. [1] / Model Component	Explanatory Variable	Par.[1] Est.	SE[1]	Z-value (Wald Statistic)	*P* Value	Stand. Par. Est.[1]
Density of Infected Hosts ~	**Transmission Coefficient, *β***	**0.060**	**0.021**	**2.829**	**0.005**	**0.415**
	Competitor/Diluter Density	**-0.015**	**0.005**	**-2.855**	**0.004**	**-0.168**
Comp./Diluter Density ~	**Growth Rate Low Res.**	**-0.415**	**0.176**	**-2.357**	**0.018**	**-0.309**
Modeled Covariances:	Transmission Coeff., *β* ~~ Growth Rate Low Res.	103.7	72.44	1.432	0.152	0.580
Intercepts:	Density of Infected Hosts	0.236	0.800	0.295	0.768	0.136
	Competitor/Diluter Density	71.69	25.25	2.839	0.005	3.583
	Transmission Coefficient, *β*	33.70	4.587	7.347	0.000	2.812
	Growth Rate Low Resources	142.4	5.634	25.28	0.000	9.540
Variances:	Density of Infected Hosts	2.340	0.815			0.774
	Competitor/Diluter Density	362.0	191.1			0.904
	Transmission Coefficient, *β*	143.6	45.38			1.000
	Growth Rate Low Resources	222.9	90.49			1.000

[1] Key to abbreviations: Dep. Var. = dependent variable; Par. Est. = parameter estimate; SE: = Standard error; Stand. = standardized

Table S5. Parameters for the path model in Fig. 6A. Bold lines indicate significant or trending relationships.

Dep. Var. [1] / Model Component	Explanatory Variable	Par.[1] Est.	SE[1]	Z-value (Wald Statistic)	*P* Value	Stand. Par. Est.[1]
Infection Prevalence ~	Focal Host Density	-0.006	0.027	-0.221	0.825	-0.033
	Competitor/Diluter Density	**-0.026**	**0.014**	**-1.901**	**0.057**	**-0.161**
	Transmission Coefficient, *β*	**0.126**	**0.045**	**2.815**	**0.005**	**0.461**
Focal Host Density ~	**Competitor/Diluter Density**	**-0.373**	**0.114**	**-3.277**	**0.001**	**-0.401**
Modeled Covariances:	Transmission Coeff., *β* ~~ Competitor/Diluter Dens.	-37.33	34.04	-1.097	0.273	-0.156
Intercepts:	Infection Prevalence	0.132	2.122	0.062	0.950	0.040
	Focal Host Density	61.27	5.268	11.629	0.000	3.291
	Competitor/Diluter Density	12.62	3.880	3.253	0.001	0.631
	Transmission Coefficient, *β*	33.70	4.587	7.347	0.000	2.812
Variances:	Infection Prevalence	8.019	2.385			0.743
	Focal Host Density	291.0	106.4			0.839
	Competitor/Diluter Density	400.3	216.3			1.000
	Transmission Coefficient, *β*	143.6	45.38			1.000

[1] Key to abbreviations: Dep. Var. = dependent variable; Par. Est. = parameter estimate; SE: = Standard error; Stand. = standardized

Table S6. Parameters for the path model in Fig. 6B. Bold lines indicate significant or trending relationships.

Dep. Var. [1] / Model Component	Explanatory Variable	Par.[1] Est.	SE[1]	Z-value (Wald Statistic)	*P* Value	Stand. Par. Est.[1]
Density of Infected Hosts ~	**Focal Host Density**	**0.040**	**0.018**	**2.233**	**0.026**	**0.438**
	Competitor/Diluter Density	0.000	0.007	-0.022	0.983	-0.002
	Transmission Coefficient, *β*	**0.054**	**0.021**	**2.614**	**0.009**	**0.380**
Focal Host Density ~	**Competitor/Diluter Density**	**-0.373**	**0.114**	**-3.277**	**0.001**	**-0.401**
Modeled Covariances:	Transmission Coeff., *β* ~~ Competitor/Diluter Dens.	-37.33	34.04	-1.097	0.273	-0.156
Intercepts:	Density of Infected Hosts	-2.020	1.454	-1.389	0.165	-1.182
	Focal Host Density	61.27	5.268	11.63	0.000	3.291
	Competitor/Diluter Density	12.62	3.880	3.253	0.001	0.631
	Transmission Coefficient, *β*	33.70	4.587	7.347	0.000	2.812
Variances:	Density of Infected Hosts	1.875	0.399			0.642
	Focal Host Density	291.0	106.4			0.839
	Competitor/Diluter Density	400.3	216.3			1.000
	Transmission Coefficient, *β*	143.6	45.38			1.000

[1] Key to abbreviations: Dep. Var. = dependent variable; Par. Est. = parameter estimate; SE: = Standard error; Stand. = standardized.

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Chapter 4

Rapid evolution buffers densities of hosts during epidemics and maintains the dilution effect

Citation:

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CHAPTER 4 ABSTRACT

Friendly competition represents an important frontier for dilution effect research. It combines two common dilution mechanisms, encounter reduction and host regulation, and produces a diverse variety of outcomes. Although focal host density is constrained by the combined threats of competition and disease, rapid host evolution could alleviate this constraint and transform costs and benefits of friendly competition. We manipulated standing trait variation and strengths of selection in a mesocosm experiment lasting 7-10 focal host (*Daphnia*) generations. Epidemics accelerated rapid host evolution of higher competitive ability, but not lower disease risk. In turn, higher competitive ability buffered focal host density from impacts of disease and competition, and especially both together. Finally, competitor/diluters still reduced infection prevalence and density of infected hosts, although evolution of competitive ability simultaneously elevated the density of infected hosts. This suite of outcomes provides divergent implications for management of zoonotic diseases and conservation of focal host density.

INTRODUCTION

Disease ecology research is moving beyond a phenomenological view of the dilution effect (meta-analysis: Civitello *et al.* 2015) and towards a predictive framework linking species diversity to disease (Keesing *et al.* 2006; Johnson *et al.* 2015). As a pattern, the dilution effect links losses of species diversity with increases in disease risk for a focal host species (Ostfeld & Keesing 2000, 2012). A more mechanistic interpretation explains which 'diluter' taxa reduce disease, why they are lost from communities as diversity declines, and how they interfere with disease transmission (Ostfeld & Keesing 2012). After all, a dilution effect ultimately results from local interactions among focal hosts, parasites, and diluters. For example, diluters can consume infectious free-living parasites, thereby reducing encounters between focal hosts and parasites (Johnson *et al.* 2010). Diluters can also compete with focal hosts, regulate their population density, and hence inhibit density-dependent disease transmission (Keesing *et al.* 2006; but see Strauss et al. in prep.). These two dilution mechanisms (encounter reduction and host regulation) operate together in the general "friendly competition" module (Hall *et al.* 2009a). Insights from friendly competition delineate specifically when competitor/diluters reduce disease, and hence ground the dilution effect in mechanistic species interactions (Strauss *et al.* 2015; Strauss et al. in prep.). Extensions of the friendly competition framework, even incorporating rapid host evolution, will further expand the predictive frontier of dilution effect research.

Without any evolving host traits, density of focal hosts in friendly competition could be dramatically lowered by the dual threats of competition and disease (Strauss *et al.* 2015). Decreases in density may intensify for focal hosts that compete weakly (and become competitively excluded) or suffer high disease risk (and become overrun with virulent infection). In principle, "friendly" competitor/diluters could net-benefit focal host

fitness during epidemics, if fitness gains (via disease dilution) outweigh losses (via competition). However, the strength of disease dilution is highest when focal hosts compete weakly (since competitor/diluters become more numerous) and suffer higher disease risk (since resistant diluters have greater relative impacts on transmission) (Strauss et al. in prep.). Thus, traits that promote dilution align precisely with traits that imperil density of focal hosts from competition and disease. Maximizing host density and minimizing infection prevalence could be valuable when focal hosts are crops (Boudreau 2013), livestock (Huang *et al.* 2013), or declining native (Thieltges *et al.* 2009) or charismatic taxa (Johnson *et al.* 2013; Venesky *et al.* 2014). In these scenarios, friendly competition may impose an undesirable constraint. However, for zoonotic diseases like Lyme disease (Ogden & Tsao 2009), hantavirus (Suzan *et al.* 2009), and schistosomiasis (Johnson *et al.* 2009), density of infected hosts can determine disease risk for humans. Here, increased benefits of dilution could warrant (or even require) lower densities of focal hosts. Thus, implications of the constraint imposed by friendly competition may vary by perspective.

Rapidly evolving focal hosts could circumvent the ecological constraint of friendly competition. Broadly, interspecific competition can select for traits that increase competitive ability (Vellend 2006; Rowe & Leger 2011; terHorst 2011). In turn, rapid evolution of competitive ability can rescue populations when invaded by superior competitors (see Strauss *et al.* 2006). Epidemics could even accelerate the evolution of higher competitive ability, since superior competitors could better compensate higher death (due to parasite virulence) by most efficiently converting resources into births (see Zbinden *et al.* 2008; Turcotte *et al.* 2011). In friendly competition, rapid evolution of competitive ability driven by competitors and/or parasites could buffer focal host density from negative impacts of competition and disease. Moreover, if competitor/diluters

remain sufficiently numerous and/or disease risk remains sufficiently high, they could still reduce disease (Strauss et al. in prep.). Thus, this eco-evolutionary feedback could simultaneously buffer focal host density from impacts of competition and disease while maintaining benefits of the dilution effect.

However, rapid host evolution in friendly competition could also increase or decrease disease, undermine the dilution effect, or reinforce competitive exclusion of focal hosts – the possibilities abound. If evolving focal host populations achieve higher densities during epidemics, they could also maintain higher densities of infections or infection prevalence (Anderson & May 1981; but see Civitello *et al.* 2013). Furthermore, focal hosts could also evolve lower disease risk during epidemics (Altizer *et al.* 2003; Penczykowski *et al.* 2011). Although evolution of lower disease risk could reduce disease while diluters are absent, it could also undermine the dilution effect if focal hosts become too resistant, and competitor/diluters become irrelevant for transmission (Strauss et al. in prep.). Finally, evolving host populations could face a tradeoff between competitive ability and disease risk (Kraaijeveld & Godfray 1997; Duncan *et al.* 2011; Duffy *et al.* 2012). If focal hosts evolve lower disease risk but weaker competitive ability, focal hosts could become competitively excluded, disease could decline, and the dilution effect could fade. On the other hand, if focal hosts become stronger competitors despite evolving higher disease risk, they could maintain robust densities and still benefit from disease dilution, despite increased density of infected hosts and/or infection prevalence. Implications of these eco-evolutionary outcomes vary starkly by perspective (zoonotic disease vs. conservation of focal host density).

Despite recently emerging ecological foundations (Strauss *et al.* 2015; Strauss et al. in prep.), this eco-evolutionary frontier for friendly competition remains completely unexplored. Rapid evolution could buffer density of focal host populations or not,

increase or decrease disease, and undermine or maintain the dilution effect. Outcomes should depend on standing trait variation in competitive ability and disease risk, relative strengths of selection imposed by competitors and parasites, and covariation among focal host traits (Day & Gandon 2007). Here, we explore outcomes of eco-evolutionary friendly competition in a mesocosm experiment lasting 7-10 focal host generations. First, we manipulate standing trait variation in focal host populations. The we track how disease and competition drive changes in genotype frequencies and mean traits of focal hosts (i.e., evolution); how mean traits regulate densities of focal hosts, their infections, and the dilution effect (i.e., ecology); and how standing trait variation and covariation fuels or constrains any eco-evolutionary dynamics. We expand this frontier of dilution effect research by revealing costs and benefits of rapid evolution in the friendly competition module.

MATERIALS AND METHODS

Natural History of the Study System

The focal host here, the cladoceran *Daphnia dentifera*, dominates grazer communities in many North American lakes (Tessier & Woodruff 2002), and frequently suffers autumnal epidemics caused by the virulent fungus *Metschnikowia bicuspidata* (Hall *et al.* 2010b; Strauss *et al.* 2016). Focal hosts incidentally consume infectious spores while filter-feeding (Hall et al. 2007). Infected hosts cannot recover, suffer decreased birth and elevated death rates (Hall *et al.* 2009b), and release spores after death. Focal host generation time ranges 7-10 days. They can evolve lower disease risk during large epidemics (via clonal selection), but higher risk during small epidemics (Duffy *et al.* 2012). This paradox likely stems from a foraging-based tradeoff, which links

high disease risk with superior resource acquisition (Hall *et al.* 2010a; Auld *et al.* 2013) and perhaps competitive ability. The competitor/diluter, the cladoceran *Ceriodaphnia sp.*, frequently reduces the size of focal host epidemics in experiments (Strauss et al. in prep) and the field (Strauss *et al.* 2016). They inadvertently consume fungal spores while foraging, rarely become infected, and hence reduce encounters between focal hosts and parasites (Hall *et al.* 2009a; Strauss *et al.* 2015). They also compete with focal hosts for resources, lower their density, and hence inhibit density-dependent disease transmission (Strauss *et al.* 2015; Strauss et al. in prep.). These interactions exemplify friendly competition.

Focal Host Traits & Populations

Previously, we measured variation in two key traits—competitive ability and disease risk—among 8 focal host isoclonal lines (Strauss et al. in prep.). Trait measurements here follow identical methods (see "Trait Measurements" in Appendix S1 in Supporting Information for details). In short, we estimated an index of disease risk (i.e., the transmission coefficient β) by fitting a mathematical model to infection assays (e.g., Hall *et al.* 2007; Hall *et al.* 2012). Fifteen individuals were exposed to each of three parasite concentrations, maintained individually, and later inspected for signs of infection. We also estimated an index of competitive ability by calculated mass accrual of juveniles while feeding on low resources (Lampert & Trubetskova 1996; Hall *et al.* 2012). In this growth rate assay, we dried and weighed body mass of individuals at birth (mean N = 9.3) and 5-6 days later (mean N = 16.6), and estimated their log mean mass accrual over time. We bootstrapped standard errors for both traits with R (R Development Core Team 2008).

In the previous experiment (Strauss et al. in prep.), variation in these traits predicted experimental outcomes of friendly competition. Strongly competing focal hosts constrained densities of competitor/diluters, remained numerous themselves, and benefited little from disease dilution. In contrast, weakly competing focal hosts benefited relatively more from disease dilution (since competitor/diluters were more numerous), but were outcompeted and sometimes nearly driven extinct. Simultaneously, focal hosts with high disease risk spread large epidemics but benefited more from disease dilution (i.e., diluters exerted larger reductions in infection prevalence during larger epidemics). In contrast, focal hosts with low disease risk spread smaller epidemics, but benefited less from disease reduction via dilution. These results provide the ecological foundation for our current eco-evolutionary experiment.

We manipulated standing trait variation by forming diverse and constrained populations of focal hosts from these previously studied isoclonal lines (Strauss et al. in prep.). Diverse populations included all lines and spread a broad range of competitive ability and disease risk (Fig. 1). Hereafter, we refer to each distinct isoclonal line as a "genotype." One "genotype" included a pair of isoclonal lines that proved indistinguishable with our microsatellite markers (see "Genotyping & Mean Traits" in Methods, below). Diverse populations also included three additional genotypes that grew poorly in the previous experiment (one also included in constrained populations). These inconsequential genotypes remained extremely rare here as well, so we ignore their traits (since they would inconsequentially change mean trait values). Constrained populations included a specific subset of three focal host genotypes with moderate competitive ability and disease risk (Fig. 1). Thus, diverse populations featured high standing trait variation to fuel eco-evolutionary dynamics, while constrained populations with low standing trait variation served as eco-evolutionary controls.

Mesocosm Experiment & Mean Focal Host Traits

Our mesocosm experiment crossed standing trait variation in the focal host population (diverse [+V] or constrained [-V]) with presence/absence of competitor/diluters (+/- C) and presence/absence of parasites (+/- P). Thus, focal host populations experienced selection imposed by competitors alone, parasites alone, neither, and both. All treatments were replicated 5 times and housed in 75-liter tanks (see "Mesocosm Experiment" in Appendix S1 for details). Nitrogen, phosphorus, and light stimulated algal growth. We added focal hosts (mean density 2.1 L^{-1} per genotype) and a single genotype of competitor/diluters (2.1 L^{-1}) on day 0. Constrained treatments began at lower overall focal host density (8 vs. 21 L^{-1}), but quickly approached densities of diverse treatments. We sampled weekly for three weeks (mixing and sieving 1 L per tank), added parasites (5,000 L^{-1}) on day 21, and then continued sampling biweekly until day 70 (7-10 focal host generations in total). We used microscopes to count samples and track changes in density of focal hosts and competitor/diluters, density of infected hosts, and infection prevalence (diagnosing infections visually at 50X). All counted samples were preserved in 70% ethanol with 5% 0.5 mM EDTA and stored at 2 degrees C for genotyping.

We documented changes in genotype frequencies and mean focal host traits both before (days 0-25) and during epidemics (days 25-70). Initial genotype frequencies were estimated from starting densities of each focal host genotype (see "Mesocosm Experiment" in Appendix S1). Later, we genotyped ~10 individuals per tank (718 total), sampled both immediately before epidemics began (day 25) and at the end of the experiment (day 70). In summary, we digested and extracted DNA, amplified microsatellite loci, determined alleles with fragment analysis, and identified genotypes by comparing alleles with cultures maintained in the laboratory (see "Genotyping" in Appendix S1 for details). Finally, we estimated mean traits of focal hosts as averages of

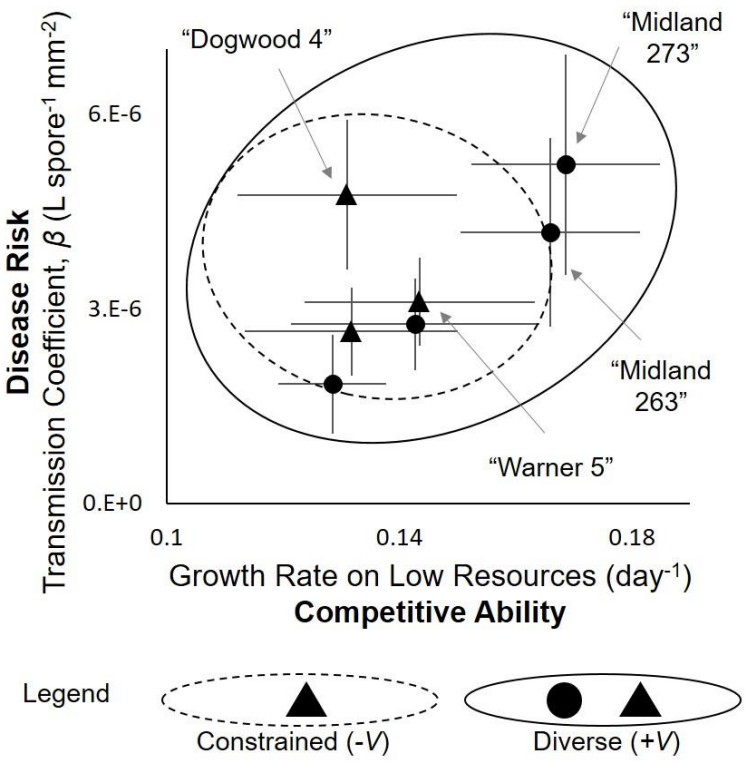

Figure 1. *Focal host genotypes vary in two key traits and form populations with constrained or diverse standing trait variation.* **Two key traits:** Growth rate on low resources (x-axis) indexes competitive ability. The transmission coefficient *β* (y-axis) indexes disease risk. Error bars are bootstrapped standard errors around each focal host genotype. **Constrained** populations (dashed ellipse outline; *-V*) contain three focal host genotypes (triangles) with moderate competitive ability and disease risk. Standing trait variation is constrained. **Diverse** populations (solid ellipse outline; *+V*) contain the same three genotypes (triangles) plus four more (circles). These additional genotypes spread the range of competitive ability and disease risk, increasing standing trait variation. Specific genotypes are identified by name for later comparisons (Figs. 3 & S2). Three inconsequential genotypes are not pictured (one in both constrained and diverse populations; two in diverse populations only). Key to abbreviations: *V* = standing trait variation.

each genotype's traits (see "Trait Measurements" in Appendix S1), weighted by genotype frequencies on days 0, 25, and 70.

Statistics

Ecology (Fig. 2): All statistical analyses were conducted in R (R Development Core Team 2010). We tracked changes in log-transformed densities of focal hosts and

competitor/diluters with repeated measures mixed models (RMMMs), using the NLME package (Pinheiro & Bates 2000). For RMMMs predicting density of focal hosts, we fit separate models for each level of trait diversity, to avoid complicated four-way interactions. However, an ANOVA confirmed that focal host densities were similar between diversity treatments before epidemics began (day 25). We also divided the time series into four periods (I-IV), to specify when treatments diverged. RMMMs included tank as a random effect (intercept only), different variances for experimental groups, and autocorrelated errors (one sampling period). Crossed fixed effects included time (*t*), presence of competitor/diluters (*C*), and presence of parasites (*P*; only after adding spores). One fixed effect differed in RMMMs predicting density of competitor/diluters: standing trait variation in the focal host population (*V*) replaced presence of competitor/diluters. Finally, we summarized epidemic size for each tank by integrating the area under the time series tracking density of infected hosts and infection prevalence. ANOVAs tested whether competitor/diluters (*C*), standing trait variation (*V*), or their interaction (*C* x *V*) impacted either metric of epidemic size.

Evolution (Figs. 3, S1 & S2): Next, we characterized changes in genotype frequencies (4 dominant genotypes and all others pooled together) and mean traits (competitive ability and disease risk), both before and during epidemics. These RMMMs included tank as random and time (*t*) as a fixed effect. To conserve statistical power, we used likelihood ratio tests to determine which other fixed effects improved model fits (see "Evolutionary Statistics" in Appendix S1 for details). We conditionally added standing trait variation (*V*), presence of competitor/diluters (*C*), and/or presence of parasites (*P*) as crossed fixed effects.

Eco-Evolutionary Impacts (Fig. 4, 5 & S3): Finally, we tested how final traits of focal hosts impacted final ecological outcomes, using generalized least squares (GLS) linear models, fit with flexible variance functions in NLME. When focal hosts were driven

extinct before day 70, we substituted traits from day 25. Final ecological outcomes (density of focal hosts, density of infected hosts, and infection prevalence) were integrated over the final period (~3 focal host generations). We tested how final traits impacted final densities of focal hosts in treatments with competition alone (+*C; -P*), disease alone (-*C; +P*), neither (-*C; -P*), and both (+*C; +P*). Without competition or disease, final focal host density remained higher in constrained the diverse populations (confirmed with a t-test). To assess whether final traits buffered focal host density from competition or disease, we standardized their absolute densities relative to these different baselines. Then, we tested whether final host traits (an index of evolution) buffered these relative (as well as absolute) host densities (an index of ecology) from competition and/or disease. Lastly, we tested whether either metric of disease, integrated over the final period, was regulated by presence of competitor/diluters (*C*) or final competitive ability of focal hosts (*CA*). Likelihood ratio tests (LRTs) determined whether an interaction between these variables (*C* x *CA*) improved model fit of linear GLS models, and hence whether evolution of host traits undermined the dilution effect.

RESULTS

Ecology (Fig. 2): Competition and disease dramatically lowered density of focal hosts in constrained populations, but densities in diverse populations were much more robust. Here, we report all significant P values from repeated measures mixed models (RMMMs). In constrained populations (Fig. 2A), density started lower, but increased during period I (*t* effect: $P < 0.001$). The complementary ANOVA confirmed that these densities were indistinguishable from diverse populations on day 25 ($P = 0.86$). Then, during period II, densities in constrained populations continued to increase in the absence of competition (*t* effect: $P < 0.001$), but less steeply (or began to decrease) in competition treatments (*C* x *t* effect: $P = 0.031$). By period III, density was lower in

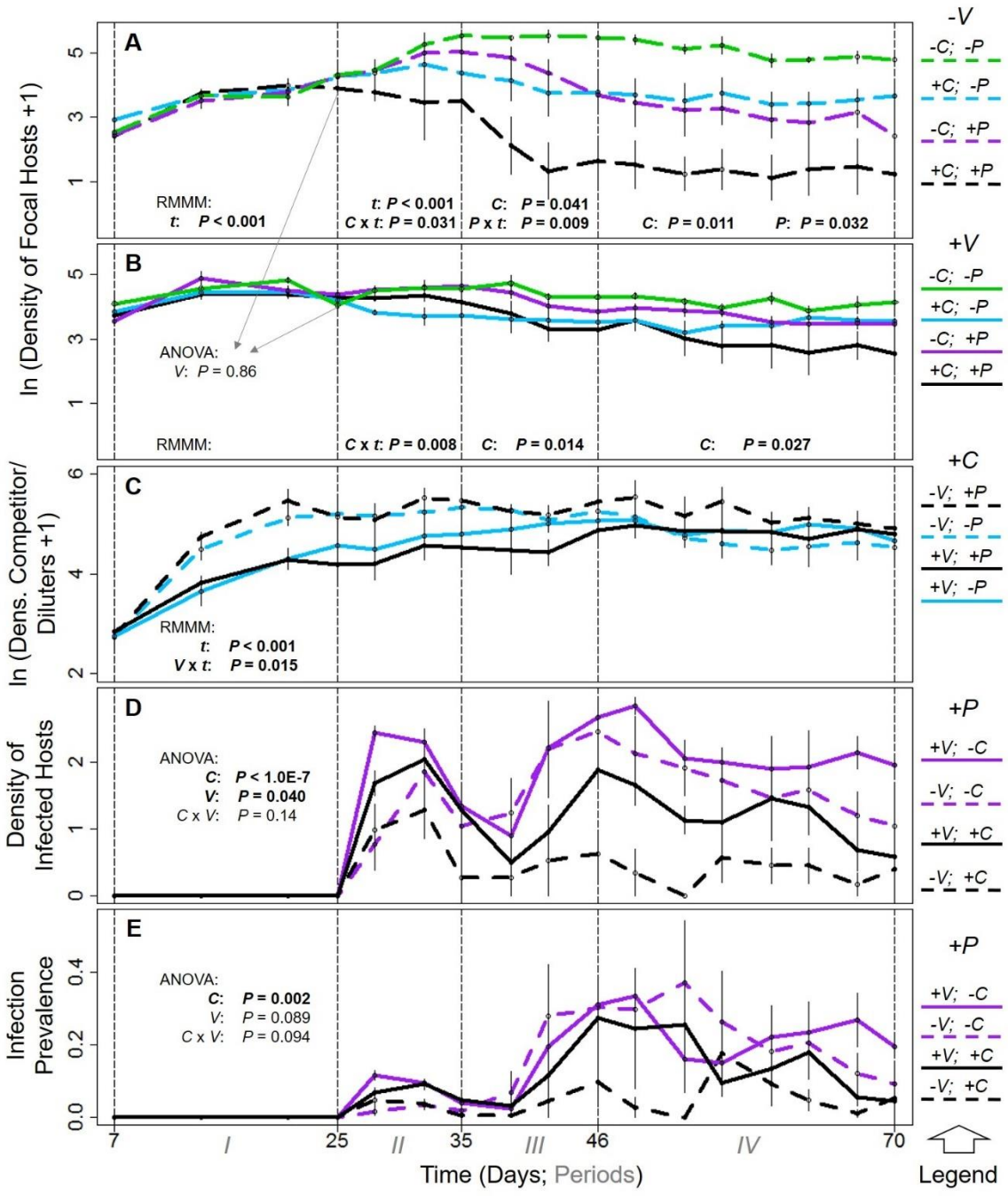

Figure 2. *Ecological dynamics unfold over four time periods (I-IV).* **A)** Density of focal hosts in constrained populations increases during period I, decreases with competition during period II, decreases with disease during period III, and remains dramatically lower with competition and disease during period IV. **B)** Density in diverse populations is stable during I, decreases with competition during II, and remains lower with competition during III and IV. **C)** Competitor/diluter density increases in I (faster in constrained

populations) and then remains stable. **D)** Integrated density of infected hosts throughout II-IV is lowered by competitor/diluters and elevated by high standing trait variation. **E)** Integrated Infection prevalence is only lowered by competitor/diluters. Key to treatments: green = focal hosts alone; purple = with parasites; blue = with competitor/diluters; black = with parasites & competitor/diluters. Abbreviations: *t* = time; *C* = competitor/diluters; *P* = parasites; *V* = standing trait variation. Error bars are standard errors.

competition treatments (*C* effect: P = 0.041), and began to decline in parasite treatments (*P* x *t* effect: P = 0.009). Throughout period IV (~3 focal host generations), densities in constrained populations remained dramatically reduced by both competition (*C* effect: P = 0.011) and disease (*P* effect: P = 0.032). In contrast, in diverse populations (Fig. 2B), density of focal hosts remained stable during time I, decreased in competition treatments during II (*C* x *t* effect: P = 0.008), and remained slightly lower in competition treatments throughout III and IV (*C* effects: P = 0.014 & 0.027, respectively).

In addition, density of competitor/diluters initially increased (Fig. 2C; *t* effect: P < 0.001), although faster in constrained treatments (*V* x *t* effect: P = 0.015). Throughout II-IV, it remained similar and unchanging among all treatments. Presence of competitor/diluters reduced the integrated density of infected hosts (Fig. 2D; *C* effect: P < 1E-7), while high standing trait variation increased it (*V* effect: P = 0.04), although trait variation did not mediate this dilution effect (*C* x *V* effect: P = 0.14). Competitor/diluters also reduced integrated infection prevalence (Fig. 2E; *C* effect: P = 0.002). However, standing trait variation neither significantly impacted infection prevalence (*V* effect: P = 0.089) nor mediated this dilution effect (*C* x *V* effect: P = 0.094).

Evolution (Figs. 3, S1 & S2): Four genotypes dominated focal host populations: “Midland 273”, “Warner 5”, “Dogwood 4”, and “Midland 263” (labeled on Fig. 1). We binned all other rarer genotypes together in a category of “Others,” which decreased in

frequency both before and during epidemics (Fig. S1 in Appendix S2; both *t* effects: $P < 0.0001$). The three inconsequential genotypes with unknown traits (included in "Others") represented only 7% of individuals identified, including 3% identified from day 70. Initial genotype frequencies appeared relatively unimportant, since the genotype with highest initial mean frequency (19%) represented only 1% of individuals identified from day 70. In contrast, the genotype which dominated diverse populations at the end of the experiment ("Midland 273"; 55%) started at a low initial frequency (6%). Thus, we focus on the four dominant genotypes and how selection on their traits may have shaped the rapid evolution of focal hosts.

Disease drove changes in genotype frequencies and competitive ability (Fig. 3). Frequency of "Midland 273" (the strongest competitor) increased slowly before epidemics, (Fig. 3A; *t* effect: $P = 0.039$), but then parasites dramatically accelerated its increase during epidemics (Fig. 3B; *P* x *t* effect: $P < 0.0001$). Similarly, frequency of "Warner 5" (the strongest competitor in constrained populations) decreased before epidemics (Fig. 3C; *t* effect: $P = 0.037$), especially in constrained treatments (*V* x *t* effect: $P < 0.0001$), but then increased in these populations during epidemics (Fig. 3D; *V* x *P* x *t* effect: $P = 0.016$). In contrast, frequency of "Dogwood 4" (a weaker competitor, present in both diversity treatments) initially increased (Fig. 3E; *t* effect: $P < 0.0001$), but declined or slowed as stronger competitors replaced it during epidemics (*P* x *t* effect: Fig. 3F; $P = 0.0036$).

These changes in genotype frequencies drove evolution of higher competitive ability during epidemics, especially in diverse populations. Mean competitive ability started slightly higher in diverse populations (Fig. 3G; *V* effect: $P < 0.0001$) and diverged further from constrained populations before epidemics (*V* x *t* effect: $P = 0.011$). Then, during epidemics, parasites drove rapid evolution of higher competitive ability in both treatments (Fig. 3H; *P* x *t* effect: $P < 0.0005$), but especially in diverse populations (*V* x *P*

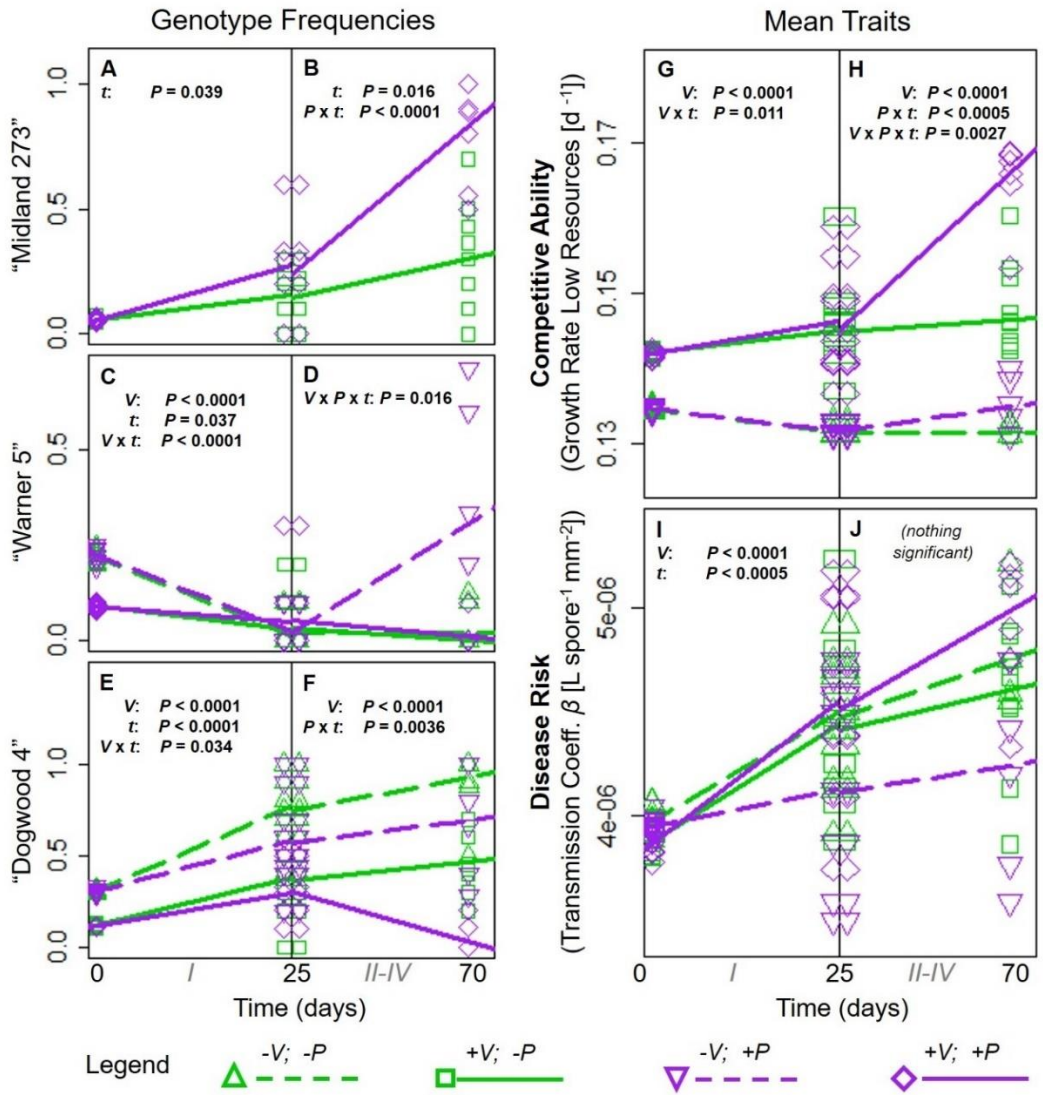

Figure 3. *Disease drives changes in genotype frequencies and elevates mean competitive ability.* P values (RMMMs) indicate all significant changes before epidemics (time I) and during epidemics (time II-IV). **Genotype frequencies:** Frequency of "Midland 273" (only present in diverse treatments) **A)** initially increases slowly. Then, **B)** epidemics accelerate its increase. Frequency of "Warner 5" **C)** initially decreases in both diversity treatment, but **D)** increases during epidemics in constrained treatments. Frequency of "Dogwood 4" **E)** initially increases, but **F)** becomes replaced by stronger competitors during epidemics. **Mean traits:** Competitive ability **G)** begins higher and initially increases in diverse treatments. Then, **H)** epidemics accelerate the evolution of higher competitive ability, especially in diverse treatments. In contrast, **I)** disease risk initially increases, and **J)** does not ultimately differ among treatments. Key to abbreviations: *t* = time; *P* = parasites; *V* = standing trait variation.

x *t* effect: P = 0.0027). Disease risk increased in both treatments before epidemics (Fig. 3I; *t* effect: P < 0.0005). However, neither parasites nor trait variation significantly impacted disease risk during epidemics (Fig. 4J). Thus, selection to become a stronger competitor not only outweighed selection to resist disease, but epidemics accelerated the evolution of higher competitive ability.

Interspecific competition also drove subtle changes in genotype frequencies and competitive ability (Fig. S2). Frequency of "Midland 263" (the second strongest competitor) initially increased in competition treatments (Fig. S2A; *C* x *t* effect: P = 0.067), and became higher in these treatments as epidemics began (Fig. S2B; *C* effect: P = 0.045). In turn, mean competitive ability initially increased in competition treatments, although only in diverse populations (Fig. S2C; *V* x *C* x *t* effect: P = 0.024). However, during epidemics, impacts of competition on competitive ability (Fig. S2D) became statistically overwhelmed by the strong impacts of disease (Fig. 3H).

Eco-Evolutionary Dynamics (Figs. 4, 5 & S3): Rapid evolution of competitive ability buffered focal host densities from competition and disease (Fig. 4). In the absence of competitor/diluters or parasites, higher final competitive ability (the index of evolution) decreased final focal host density (Fig. S3A; P < 0.001). In other words, constrained populations maintained higher baseline densities than diverse populations (Fig. 4A; t-test; P = 0.0024). After standardizing densities by these inherently different baselines between diversity treatments, higher final competitive ability of focal hosts clearly buffered focal host density from competition with competitor/diluters (Fig. 4B; P = 0.029), disease alone (Fig. 4C; P = 0.0011), and both interspecific competition and disease together (Fig. 4D; P = 0.0037). In each case, density scaled to the baseline (Fig. 4A) increased with evolved competitive ability of focal hosts; hence, rapid trait evolution "buffered" focal host density. Before scaling these densities appropriately, evolution of competitive ability did not appear to impact focal host density in treatments

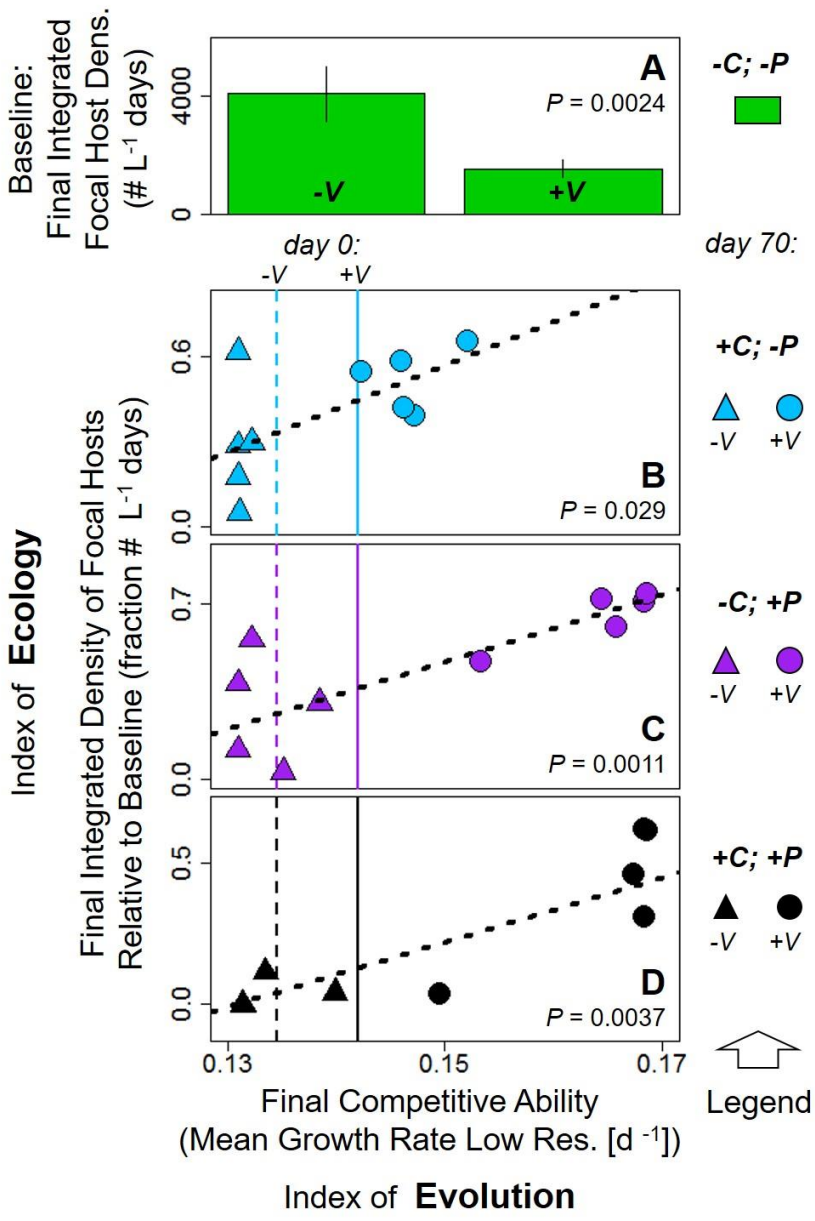

Figure 4. *Rapid evolution of higher competitive ability buffers densities of focal hosts from competition and disease.* **A)** In the absence of competitor/diluters and parasites, focal hosts maintain higher baseline densities in constrained populations (integrated over time IV; see Fig. 2). **B-D)** Final mean competitive ability (indices of evolution; from day 70) are plotted on x-axes (triangles = constrained [*-V*]; circles = diverse [*+V*]). Final focal host densities (indices of ecology) are integrated over time IV and standardized relative to baseline (baseline =1 on these scaled y-axes). Vertical lines indicate initial mean competitive ability (dashed = constrained; solid = diverse). In treatments with **B)** only parasites (*-C; +P*), **C)** only competitor/diluters (*+C; -P*), and **D)** both (*+C; +P*), higher final competitive ability buffers densities of focal hosts relative to baseline. Absolute density also increases with competitive ability in treatments with both competition and disease (Fig. S3D). Legend follows Fig. 2.

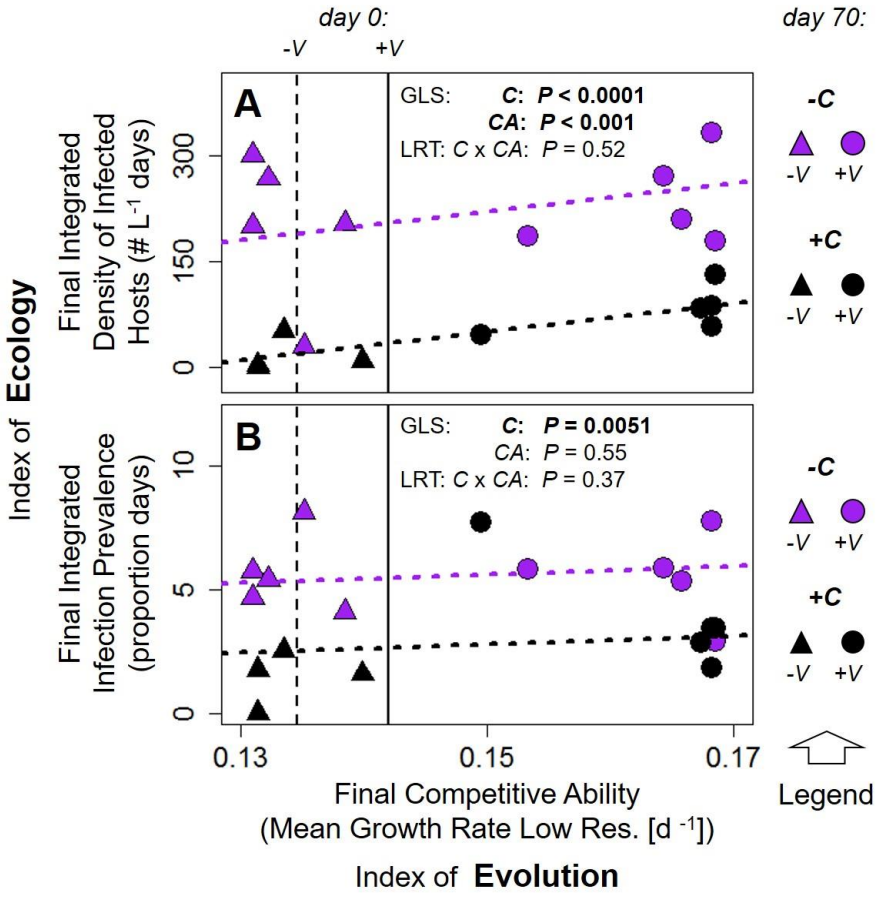

Figure 5. *Rapid evolution of higher competitive ability maintains the dilution effect but elevates density of infected hosts.* Vertical lines indicate initial mean competitive ability (dashed = constrained [-*V*]; solid = diverse, [+*V*]). Final mean competitive ability is plotted (from day 70: triangles = constrained; circles = diverse). Both final density of infected hosts and infection prevalence are integrated over time IV (see Fig. 2). **A)** Competitor/diluters lower final density of infected hosts (*C* effect). Simultaneously however, higher final competitive ability increases it (*CA* effect). **B)** Competitor/diluters also lower final integrated infection prevalence (*C* effect), but final competitive ability has no impact (*CA* effect). Importantly, evolution of competitive ability does not undermine either of these dilution effects (no *C* x *CA* interactions). Legend follows Fig. 2.

with competition or disease alone (Fig. S3 in Appendix 2), although it still raised absolute density of focal hosts in treatments with both competition and disease (Fig. S3D; P = 0.0082).

Finally, rapid evolution of competitive ability maintained the dilution effect, but increased the density of infected hosts (Fig. 5). Final integrated density of infected hosts

during period IV was lowered by presence of competitor/diluters (Fig. 5A; *C* effect: $P < 0.0001$) and raised by higher final competitive ability (*CA* effect: $P < 0.001$). Because focal hosts that evolved higher competitive ability were less strongly impacted by disease, they likely maintained higher densities of infections. However, final competitive ability did not interact with competitor/diluters' impacts on disease (*C* x *CA* likelihood ratio test: $P = 0.52$), and hence did not undermine the dilution effect. Presence of competitor/diluters also lowered final integrated infection prevalence (Fig. 5A; *C* effect: $P = 0.0051$). However, final competitive ability neither impacted this metric of disease (*CA* effect: $P = 0.55$), nor undermined this dilution effect either (*C* x *CA* likelihood ratio test: $P = 0.37$).

Discussion

Friendly competition represents an important frontier for dilution effect research. It combines two common dilution mechanisms, encounter reduction and host regulation, and delineates how and when each mechanism reduces disease (Strauss et al. in prep.). A variety of outcomes can emerge: focal hosts can be driven extinct by interspecific competition and disease, diluters can minimize infection prevalence and density of infected hosts, or focal hosts can repress diluters and spread uncontrollable epidemics (Strauss *et al.* 2015). Focal host fitness is constrained, because the strength of disease dilution is maximized by the same focal host traits that exacerbate the negative impacts of competition and disease on focal host density. Yet rapid host evolution and eco-evolutionary feedbacks could dismantle these ecological constraints and transform the costs and benefits of friendly competition. Can rapid evolution save focal host density from extinction driven by competition and disease? Can it undermine the dilution effect, or even increase disease? Even though purely ecological theory for

friendly competition is still developing, these eco-evolutionary expansions further transform this frontier of dilution effect research.

Our experiment emphasizes the double dangers of friendly competition and a potential resolution for focal hosts. Specifically, density in constrained treatments suffered severely and additively from both competition and disease (Fig. 2A). In these populations, negative impacts of competitor/diluters on focal host density vastly outweighed any benefits of disease dilution for focal hosts. On the other hand, competition still reduced densities in diverse populations, but less dramatically (Fig. 2B). Moreover, disease did not significantly impact these host densities, even though it exacted large density tolls from constrained populations. Instead, in diverse populations, rapid evolution of competitive ability of focal hosts (Fig. 3) buffered losses to competition alone (Fig. 4B), disease alone (Fig. 4C), and especially competition and disease together (Figs. 4D & S3D). In other words, standing trait variation rescued focal host density and fundamentally altered outcomes of friendly competition. Therefore, future theory for the dilution effect needs to further develop this eco-evolutionary perspective.

Rapid evolution buffered focal host density in diverse populations by increasing competitive ability, especially during epidemics. As predicted by extant theory (Vellend 2006), interspecific competition increased mean competitive ability of focal hosts in diverse populations, but only before epidemics (Fig. S2C). Less intuitively, during epidemics, the strongest competitors replaced weaker genotypes (Fig. 3F) in both diverse (Fig. 3B) and constrained populations (Fig. 3D). Thus, epidemics accelerated the evolution of higher competitive ability, especially in diverse populations (Fig. 3H). Most likely, disease accelerated clonal turnover towards increased competitive ability (see also Zbinden *et al.* 2008). A similar mechanism may also accelerate evolution between competing aphid genotypes (Turcotte *et al.* 2011). Consequently, our index of competitive ability—growth rate on low food—appears critical for maintaining robust

densities despite interspecific competition and disease. In a previous mesocosm experiment, each focal host genotype was grown individually with competitor/diluters and disease (Strauss et al. in prep.). Variation in this trait predicted mean density of competitor/diluters, which in turn predicted mean density of focal hosts (ranging 30-87 L^{-1} among genotypes). Here, these ecological benefits of higher competitive ability became apparent through an eco-evolutionary feedback. Specifically, rapid evolution of higher competitive ability buffered focal host densities in treatments with competitor/diluters (Fig. 4B), parasites (Fig. 4C), and both (Fig. 4D).

Disease did not trigger rapid evolution of lower disease risk in our experiment (Fig. 3J). This may seem surprising, since *Daphnia* can rapidly evolve resistance to various parasites (Duncan & Little 2007; Zbinden *et al.* 2008), including the fungus here (Duffy *et al.* 2012). In the previous mesocosm experiment (Strauss et al. in prep.), variation in disease risk predicted mean infection prevalence, ranging 4-17% among focal host genotypes. Thus, lower disease risk must have provided some fitness advantage. Indeed, models predict rapid evolution of resistance during epidemics (e.g., Duffy & Sivars-Becker 2007), especially when virulence is high and tradeoffs are lacking (Duffy & Forde 2009). However, in this study system, a tradeoff links lower disease risk with inferior resource acquisition (Hall *et al.* 2010a; Auld *et al.* 2013) and likely weaker competitive ability (see Fig. 1). Moreover, this parasite does not castrate (Hall *et al.* 2009b). These two features could allow a strongly competitive focal host population to 'outgrow' fitness costs of infection, especially during smaller epidemics (e.g., Duffy *et al.* 2012). In our experiment, disease risk increased before epidemics (Fig. 3I), likely because it was correlated with competitive ability (at least in diverse populations; see Fig. 3G). Then, if anything, it continued to increase during epidemics (although not significantly: Fig. 3J). This result likely hinges on virulence of the parasite, and the

tradeoff characterized by the variance and covariance between competitive ability and disease risk (Day & Gandon 2007).

Despite buffering focal host density, evolution of competitive ability did not undermine the dilution effect. Specifically, presence of competitor/diluters reduced both density of infected hosts (Fig. 2D) and infection prevalence (Fig. 2E), integrated throughout the experiment. Evolution of low disease resistance could have obviated the dilution effect, since competitor/diluters become irrelevant for disease transmission as disease risk in focal hosts declines (Strauss et al. in prep.). However, disease risk did not decline (Fig. 3I-J). Evolution of higher competitive ability could also have eroded the dilution effect, if competitor/diluters became too sparse to impact disease (Strauss et al. in prep.). However, competitor/diluters remained numerous (Fig. 2C). Furthermore, evolution of competitive ability did not undermine competitor/diluters' impacts on disease (Fig. 5). Thus, rapidly evolving hosts benefited from buffered densities and consistent disease dilution, dismantling the ecological constraint of friendly competition. This combination of results is especially encouraging in disease systems when high densities of healthy focal hosts are desirable (e.g., Boudreau 2013; Huang *et al.* 2013; Johnson *et al.* 2013). However, these twin benefits are not guaranteed, and likely depend on the variance and covariance of host traits. Nevertheless, in our study system and with our host traits, eco-evolutionary feedbacks did not undermine the dilution effect.

Although rapid evolution buffered host densities and maintained the dilution effect, it also increased the density of infected hosts (Fig. 5A). This outcome holds ominous implications for zoonotic diseases (Johnson *et al.* 2009; Ogden & Tsao 2009; Suzan *et al.* 2009), because higher densities of infected hosts may threaten humans with infection. In these cases, lowering the density of infected hosts may be a higher priority than maintaining high overall densities of focal hosts, or lowering their infection prevalence. From this perspective, our experiment uncovered tension between an

ecological force (the dilution effect, which reduced density of infected hosts), and an evolutionary force (increased competitive ability, which buffered focal host density and consequently increased density of infected hosts). Here, these two forces were roughly equal in strength (Fig. 2D). However, in other cases, with different traits or species, disease dilution or host evolution could have stronger impacts on disease. Thus, host evolution does not necessarily promote disease control, and could even overwhelm benefits of the dilution effect (although evolution of lower disease risk could present a more favorable outcome). On the other hand, when conservation of high focal host density is a priority (e.g., Boudreau 2013; Huang *et al.* 2013; Johnson *et al.* 2013), a higher density of infected hosts may represent a more reasonable cost.

Future eco-evolutionary theory for friendly competition must grapple with complexities that emerge at the intersection of host-parasite and consumer-resource dynamics. A better synthesis could help anticipate surprising results. For example, epidemics in our experiment accelerated the evolution of higher competitive ability, despite the cost (albeit weak) of higher disease risk. Our explanation involves resource exploitation of hosts during epidemics. Additionally, constrained treatments maintained higher baseline densities than corresponding diverse treatments, during the final ~3 generations of the experiment (Fig. 4A). Perhaps weaker competitors in constrained populations did not depress resources as strongly and benefited from higher primary production. Stage structure dynamics may have also contributed, since these constrained populations maintained higher percentages of juveniles (64 vs. 42%). Regardless, the difference in baseline densities between diversity treatments shaped how we evaluated “buffering” of focal host density. Impacts of evolution on absolute focal host densities did not appear as strong (Fig. S3). Yet these relationships are misleading, since they ignore important differences (i.e., different baselines between

diversity treatments) that likely arose from internal intraspecific consumer-resource dynamics. Thus, as eco-evolutionary theory for friendly competition continues to expand, it must also strengthen its foundations in underlying host ecology. More unified consumer-resource-disease theory could help predict these, and likely other, surprising results.

In summary, the combination of competition and disease can be disastrous for focal host densities. Yet standing variation in traits of focal hosts can ameliorate this dilemma, and fundamentally alter outcomes of friendly competition. Eco-evolutionary dynamics can be surprising: we detected disease-accelerated evolution of competitive ability, but no evolution of disease resistance. Rapid evolution of competitive ability simultaneously buffered focal host densities and maintained benefits of the dilution effect, but also raised density of infected hosts. Implications of this suite of outcomes may vary by perspective (from zoonotic diseases to conservation of focal host density). Further advancements should characterize the variance and covariance of focal host traits in nature and continue to synthesize eco-evolutionary disease theory and consumer-resource dynamics. Additional extensions could incorporate rapid evolution of diluter populations and integrate impacts of other parasites or predators into the friendly competition module. Thus, the eco-evolutionary dynamics of friendly competition remain an expansive frontier for dilution effect research.

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CHAPTER 4 SUPPORTING INFORMATION

Appendix S1

In this appendix, we provide additional methodological details. Specifically, we describe our trait measurement assays, setup logistics for the mesocosm experiment, and genotyping protocols. Then we describe our model selection criteria for evolutionary statistical analyses, and tabulate the results of our step-wise model selection.

Trait Measurements

Methods for trait measurements are identical to Strauss (et al. in prep.). Prior to trait measurement assays, all isoclonal lines were fed high densities of high quality laboratory-cultured algae (2.0 mg mass/L *Ankistrodesmus falcatus*). Cultures were maintained in high hardness COMBO (artificial lake water media) under ideal conditions for three generations, in order to standardize any maternal affects.

Disease Risk: We calculated an index of disease risk (the transmission coefficient, β) from infection assays. This transmission coefficient represents the probability of a focal host becoming infected, given density of infectious spores (Z), the duration of spore exposure (t), and body length of the focal host (L). Disease transmission depends on body length, because larger hosts encounter parasites at a higher foraging rate (Hall *et al.* 2007). For the assay, we first reared cohorts of neonates of each isoclonal line (fed 1.0 mg mass/L/day of highly edible algal food, *Ankistrodesmus*). After 5 days, individuals were isolated in 15 mL of media. Fifteen of these individuals were exposed to each of three densities of fungal spores (Z): 75, 200,

or 393 spores/mL (at 1.0 mg mas/L/day of algal food). Spores (< 6 weeks old) were all reared in a standard focal host genotype. After ~8 hours of exposure (*t*), we measured body length of all individuals (*L*) with a dissecting microscope and micrometer. Thereafter, we transferred each individual to a fresh 50 mL tube of media daily, until death. Dead individuals were visually inspected with the dissecting microscope in order to diagnose infection. Individuals that died too early to determine infection were omitted from the analysis. This assay was conducted in three different experimental blocks, with 2 isoclonal lines repeated among blocks, in order to control for any block effects (due to potential variation in spore infectivity).

To estimate the transmission coefficient (*β*) from this transmission assay, we simplified a previous mathematical model (e.g., Hall *et al.* 2007; Hall *et al.* 2012). This model assumes that initial density of susceptible hosts in the assay (S_i; one per tube) decreases as susceptible hosts (*S*) contact spores (*Z*) at rate βL^2, where *β* is a size-controlled transmission coefficient, and L^2 is proportional to surface area. Specifically, $\frac{dS}{dt} = -\beta L^2 SZ$. Solving this equation for the final density of susceptible hosts (S_f), after exposure time (*t*), yields: $S_f = S_i \exp(-\beta L^2 Z t)$. We estimated the transmission coefficient (*β*) for each isoclonal line, using maximum likelihood and the BBLME package in R (Bolker 2008; R Development Core Team 2010). The binomial distribution (infected or not) served as the likelihood function. After controlling for block effects, we bootstrapped standard errors for each focal host genotype.

Competitive Ability: We calculated an index of competitive ability with juvenile growth rate assays on low resources (e.g., Hall *et al.* 2012). Mass accrual of neonates during a 5-6 day juvenile period becomes directly proportional to fitness, once adults begin investing energy in reproduction (Lampert & Trubetskova 1996). In turn, competitive ability depends on fitness when resources are limiting (reviewed: Grover

1997; Strauss et al. in prep.). Thus, focal hosts with high juvenile growth rates on low food resources should become strong competitors.

To calculate juvenile growth rate, we first isolated cohorts of neonates (< 24 hours old) for each focal host genotype. We obtained initial day 0 mass measurements (m_i), by drying and weighing 6-13 neonates (mean N = 11.1 per genotype) with a Mettler microbalance (Mettler-Toledo, Columbus, Ohio, USA). We also placed 11-18 live neonates (mean N = 14.5 per genotype) in separate 50 mL tubes of media. Each day, we transferred these individuals into fresh media (fed 0.15 mg mass/L *Ankistrodesmus* daily). Then, after 5 or 6 days (*d*), we dried and weighed these individuals, yielding final mass estimates (m_f). With these data, we calculated juvenile growth rate on low resources (*GR*) as the mean for each combination of initial and final mass estimates: $GR = [\ln(m_f) - \ln(m_i)] \,/\, d$. Finally, we bootstrapped standard errors around means for each focal host genotype in R.

Mesocosm Experiment

Our mesocosm experimental design crossed standing focal host trait variation (constrained [-V] or diverse [+V]) with presence/absence of parasites (+/- P) and competitor/diluters (+/- C) . Each replicate was housed in a 75-liter acid-washed polyethylene tanks in a climate-controlled room and grown under a 16 L: 8 D light cycle. We began preparing tanks by filling them to 60 liters with high-hardness COMBO (artificial lake water). Then, we added initial doses of nitrogen and phosphorus in the form of sodium nitrate and potassium phosphate (300 ug L^{-1} N as $NaNO_3$ and 20 ug L^{-1} P as K_2HPO_4). We replaced evaporated COMBO and replenished 5% of the initial nutrient dose per day, throughout the experiment. Finally, we inoculated the tanks with 50 mg

dry weight of *Ankistrodesmus falcatus*, and let this algae grow for two days prior to adding any focal hosts.

Next, we added focal hosts to the experiment. Each focal host genotype was reared in bulk-up tanks, sampling in triplicate to determine densities, and added to appropriate experimental tanks. Genotypic identities of the eight genotypes featured in Strauss (et al. in prep.) are included here in brackets. Constrained populations received four genotypes (mean density 2.1 hosts L^{-1}; total density 8.3 hosts L^{-1}), including "Dogwood 4" [G7] (2.5 L^{-1}), "Warner 5" [G4] (1.8 L^{-1}), "Bristol 112" [G5] (2.7 L^{-1}), and "A4-5" (1.2 L^{-1}). Diverse populations received all ten genotypes (mean density also 2.1 hosts L^{-1}; total density 21 hosts L^{-1}), including "Downing 282" [G1] (1.9 L^{-1}), "Midland 273" [G8] (1.3 L^{-1}), "Midland 263" [G6] (2.3 L^{-1}), "Bristol 10" [G3] (2.2 L^{-1}), "Bristol 6" (0.9 L^{-1}), "Standard" (2.8 L^{-1}), "Dogwood 4" [G7] (2.5 L^{-1}), "Warner 5" [G4] (1.8 L^{-1}), "Bristol 111" [G2] + "Bristol 112" [G5] (4.1 L^{-1}), and "A4-5" (1.2 L^{-1}). Finally, we added competitor/diluters (single genotype; 2.1 L^{-1}) on day 0 and parasites (5,000 L^{-1}) on day 21 to appropriate experiment tanks. In one tank, we detected a large unprecedented spillover of disease into the competitor/diluter population. Since these infections fundamentally changed the ecological dynamics, we omitted this extreme outlier tank from all analyses.

Genotyping

DNA Extraction: Individuals for genotyping were selected from preserved samples. If fewer than 10 individuals were available on days 25 and 70, we genotyped all of them (mean N = 8.2 per tank, per time). Overall, we genotyped 718 individuals. None were visibly infected, and adults were selected over juveniles when possible, since they yielded more DNA. First, we rinsed each individual in deionized water to remove ethanol. Then we digested tissue and extracted DNA by grinding (automatic pestle, 10

seconds) and incubating each individual in 60 µL proteinase-K extraction buffer (protocol modified from Schwenk *et al.* 1998). The extraction buffer included 43.5 mL ddH_20, 500 µL Tris Hcl (1 M PH 8.3), 5 mL KCl (0.5 M), 250 µL 1% Tween 20, 250 µL 1% NP40, and 500 µL Proteinase K solution (20 mg/mL). The Proteinase K solution included 0.5 mL glycerol, 0.5 mL ddH_20, and 20 mg Proteinase K. After being vortexed and briefly centrifuged (1000 RPM), samples were incubated at 50 degrees C for 4 hours. After 4 hours of enzyme activity, we denatured the Proteinase K by raising the temperature to 95 degrees C (3 minutes). The resulting DNA products were then frozen and stored for PCR.

PCR & Fragment Analysis: Next, we amplified 5 microsatellite loci in our DNA samples with PCR. We used primers designed by Fox (2004), including Dgm105, Dgm106, Dgm109, Dgm112, and Dgm112. Each PCR reaction used 6 µL Qiagen multiplex PCR mastermix, 1.2 µL of primer mix (2 mmol each), 3.8 µL ddH_20, and 1 µL DNA sample. PCR was run on a SimpliAmp Thermal Cycler. Cycling conditions were initiated with one cycle at 95 °C for 15 minutes, followed by 30 cycles of (94 °C for 30 s, 58 °C for 180 s, 72 °C for 90 s) and a final extension at 72 °C for 10 minutes. Amplified DNA was diluted (1 µl amplified DNA and 10 µl ddH_20) and sent to the W.M. Keck Center for Comparative and Functional Genomics (University of Illinois at Urbana-Champaign Biotechnology Center, Urbana, IL, USA) for microsatellite fragment analysis. Alleles were called using GeneMapper™ software (Version Version 5: Applied Biosystems, Foster City, CA, USA). Finally, we identified genotypes of our samples by comparing their alleles with known alleles of our laboratory-maintained isoclonal lines.

Evolutionary Statistics

We analyzed changes in genotype frequencies (4 dominant genotypes [Figs. 3 & S2] and all others pooled together [Fig. S1]) and mean traits (competitive ability [Fig. S2] and disease risk [Fig. 3]) with repeated measures mixed models using the NLME package in R (Pinheiro & Bates 2000; R Development Core Team 2010). We fit separate models before (time period I) and during epidemics (time II-IV). Unfortunately, we could not fit comprehensive models that fully crossed standing trait variation (*V*), presence of parasites (*P*), presence of competitor/diluters (*C*), and time (*t*). Such complicated models required more parameters than could be fit with our limited data, and would have also generated complicated 4-way interactions. Thus, to both conserve statistical power and clarify interpretation of our results, we used step-wise model comparison and likelihood ratio tests to justify inclusion of each factor in the models. Thus, final models (depicted in Figs. 3, S1 & S2) only included statistically relevant predictors.

Our model selection procedure included several steps. First, we accounted for repeated measures by including tank as a random effect. All time I models (changes before epidemics) included random slopes only, since we did not empirically measure initial genotype frequencies. Instead, we estimated genotype frequencies and bootstrapped variation around them using sampling variation from bulk-up tanks (see "Mesocosm Experiment" in Appendix S1, above). Different iterations of bootstrapping did not impact model selection. In contrast, all time II-IV models (changes during epidemics) included random intercepts. Likelihood ratio tests determined whether random slopes were added as well. All models included time (t) as a fixed effect. Then, for each additional crossed fixed effect, we tested whether its inclusion improved model fit, both with and without flexible variance functions to account for heteroskedasticity

(Pinheiro & Bates 2000). Standing trait variation (V) improved nearly all models and was universally included. Last, we tested whether adding parasites (P) or presence of competitor/diluters (C) improved fits. We report P values from likelihood ratio tests at each of our model selection steps, both for models predicting genotype frequencies (Table S1) and mean focal host traits (Table S2).

For a few models, we deviated from our selection procedure due to one of three reasons. Footnotes in Tables S1 & S2 chronicle these deviations. First, parameters were included if they did improved fit in the corresponding time model, even if they did not improve fit of the current model. For example, when 'parasites' improved model fit during (but not before) epidemics, it was included in both models. These comparisons confirmed that parasites had no impact until epidemics began. Second, parameters were excluded if the more complex model did not produce any new significant terms. This procedure avoided overfitting models, and clarified our core results. Third, when inclusion of 'parasites' and 'competitor/diluters' each improved model fit, we only included one factor (not both) to avoid overfitting the model. This only occurred twice. For genotype frequency of "Midland 263" we included 'competitor/diluters' in the final model, since this factor explained evolution of competitive ability before epidemics (see Fig. S2A). For mean competitive ability, we including 'parasites' (*P*) and 'competitor/diluters' (*C*) in separate models. Competitor diluters accelerated evolution of competitive ability before epidemics (Fig. S2C), but parasites accelerated evolution of competitive ability during epidemics (Fig. 3H).

Table S1. Model comparison identifies the most important predictors of genotype frequencies. Significant P values reflect improvements in model fit, based on likelihood ratio tests. Yes/no indicates whether the parameter was included in the model.

Genotype	Time Period	Figure Panel	Random Intercept	Random Slope	St. Trait Var. (***V***)	Parasites (***P***)	Competitor/ Diluters (***C***)
"Midland 273"	*I*	*3 A*	no*	yes*	no†	*P* = 0.08; yes‡	*P* = 0.14 no
	II-IV	*3 B*	yes*	*P* < 2E-4 yes	no†	*P* < 1E-4 yes	*P* = 0.21 no
"Warner 5"	*I*	*3 C*	no*	yes*	*P* < 1E-4 yes	*P* = 0.58 yes‡	*P* = 0.47 no
	II-IV	*3 D*	yes*	*P* < 1E-4 yes	*P* = 4E-3 yes	*P* < 1E-4 yes	*P* = 0.97 no
"Dogwood 4"	*I*	*3 E*	no*	yes*	*P* < 1E-4 yes	*P* = 0.11 yes‡	*P* = 0.02 no§
	II-IV	*3 F*	yes*	*P* = 0.02 Yes	*P* < 1E-4 yes	*P* < 1E-4 yes	*P* = 0.10 no
"Midland 263"	*I*	*S2 A*	no*	yes*	no†	*P* = 0.30 no	*P* = 0.003 yes
	II-IV	*S2 B*	yes*	*P* = 0.32 no	no†	*P* = 0.03 no¶	*P* < 2E-4 yes
Others (Pooled)	*I*	*S1 A*	no*	yes*	*P* < 1E-4 yes	*P* = 0.26 no	*P* = 0.051 no
	II-IV	*S1 B*	yes*	*P* = 0.30 no	*P* = 0.36 yes‡	*P* = 0.03 no§	*P* = 0.07 no

* Time I models have random slopes but not intercepts. Time II-IV models have random intercepts and potentially have random slopes (see “Evolutionary Statistics” in Appendix S1 above for details)

† Genotype only present in diverse populations (cannot include trait variation as factor)

‡ Parameter included because it improves fit in corresponding time model (time I or II-IV)

§ Parameter excluded because no additional terms in model become significant

¶ Parameter excluded because models with both P and C are overfit.

Table S2. Model comparison identifies the most important predictors of mean focal host traits. Significant P values reflect improvements in model fit, based on likelihood ratio tests. YES/NO indicates whether the parameter was included in the model.

Traits	Time Period	Figure Panel	Random Intercept	Random Slope	St. Trait Var. (***V***)	Parasites (***P***)	Comp./ Diluters (***C***)
Competitive Ability (P)	*I*	*3 G*	no*	yes*	*P* < 1E-4 yes	*P* = 0.91 yes‡	*P* = 0.02 no¶
	II-IV	*3 H*	yes*	*P* < 2E-4 yes	*P* < 1E-4 yes	*P* < 1E-4 yes	*P* = 0.58 no
Competitive Ability (C)	*I*	*S1 C*	no*	yes*	*P* < 1E-4 yes	*P* = 0.91 no	*P* = 0.02 yes
	II-IV	*S1 D*	yes*	*P* < 2E-4 yes	*P* < 1E-4 yes	*P* < 1E-4 no¶	*P* = 0.58 yes‡
Disease Risk	*I*	*3 I*	no*	yes*	*P* < 1E-4 yes	*P* = 0.07 yes‡	*P* = 0.10 no
	II-IV	*3 J*	yes*	*P* = 0.11 no	*P* = 0.14 yes‡	*P* < 4E-3 yes	*P* = 0.28 no

* Time I models have random slopes but not intercepts. Time II-IV models have random intercepts and potentially have random slopes (see “Evolutionary Statistics” in Appendix S1 above for details)

‡ Parameter included because it improves fit in corresponding time model (time I or II-IV)

¶ Parameter excluded because models with both *P* and *C* are overfit.

Appendix S2

In this appendix, we present three supplementary figures. First, we confirm that frequencies of all rare genotypes (pooled together) decrease uniformly throughout the experiment (Fig. S1). Thus, this analysis justifies pooling them together. Then, we show how competitor/diluters increase frequency of "Midland 263" before epidemics (Fig. S2A-B), which accelerates evolution of higher competitive ability in these treatments (Fig. S2C-D). Finally, we show how final competitive ability relates to absolute final host densities (Fig. S3).

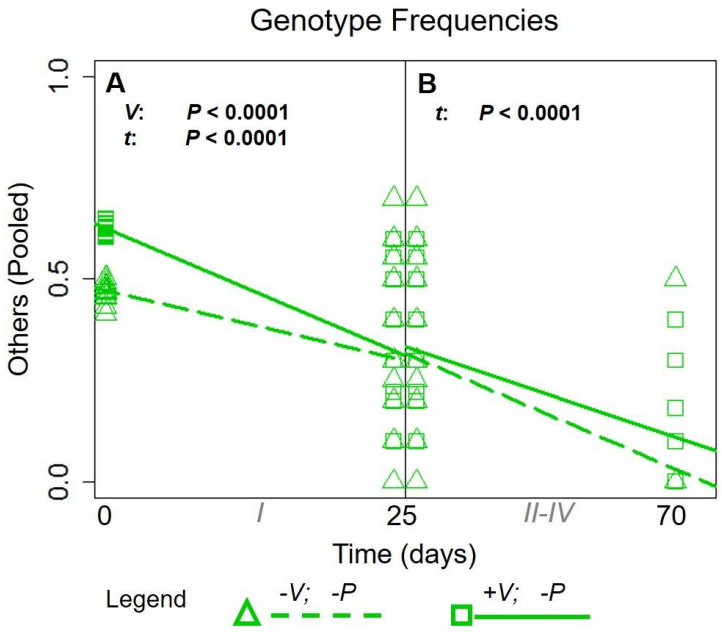

Figure S1. *Frequency of all rare genotype pooled together decreases uniformly throughout the experiment.* P values indicate all significant changes before epidemics (time I) and during epidemics (time II-IV). "Others" includes all genotypes except for "Midland 273" (Fig. 3A-B), "Warner 5" (Fig. 3C-D), "Dogwood 4" (Fig. 3E-F), and "Midland 263" (Fig. S2A-B). Frequency of all other genotypes pooled together **A)** initially starts higher in diverse populations, but decreases similarly and steeply in both diverse and constrained populations. **B)** This uniform decrease continues during epidemics. Key to abbreviations: *t* = time; *P* = parasites; *V* = standing trait variation.

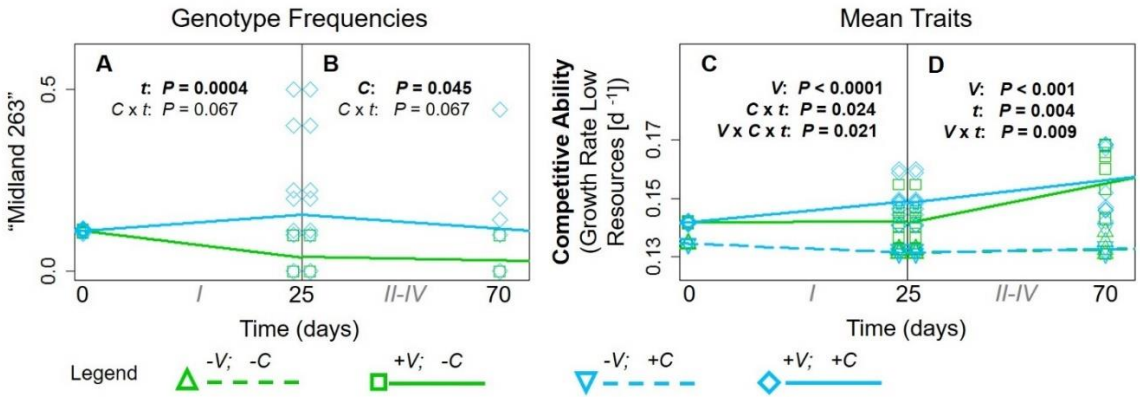

Figure S2. *Interspecific competition elevates competitive ability of focal host prior to epidemics.* **A)** Frequency of "Midland 263" (the second strongest competitor; only present in diverse treatments) initially increases in competition treatments, and **B)** becomes significantly higher in these treatments as epidemics begin. **C)** Mean competitive ability starts slightly higher in diverse treatments and initially increases in these treatments when competitor/diluters are present (in part due to frequency of "Midland 263"). **D)** During epidemics, it increases in diverse but not constrained treatments, where it remains lower. At the end of the experiment, any impacts of competition are likely statistically overwhelmed by the strong impact of disease accelerating the evolution of higher competitive ability (see Fig. 3H). Key to abbreviations: *t* = time; *C* = competitor/diluters; *V* = standing trait variation.

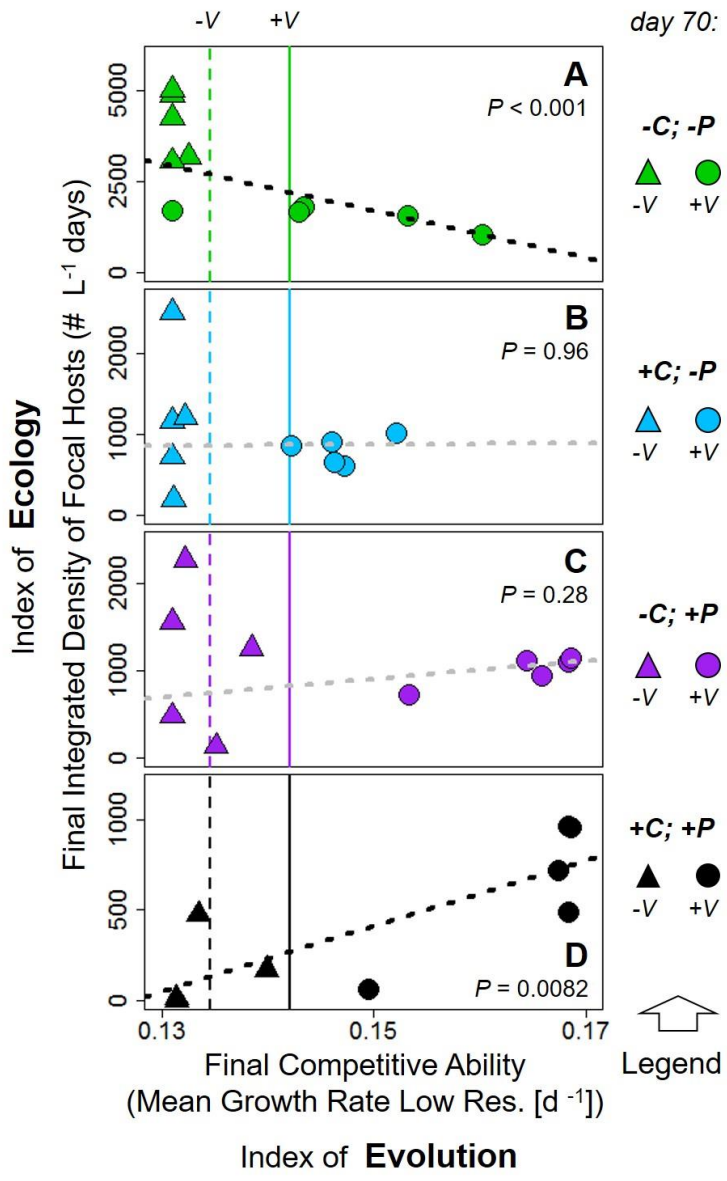

Figure S3. *Rapid evolution of competitive ability increases final absolute focal host density when challenged by both competition and disease.* Vertical lines indicate initial mean competitive ability (dashed = constrained [-*V*]; solid = diverse [+*V*]). Final mean competitive ability is plotted (triangles = constrained; circles = diverse). Final focal host density is integrated over time IV (see Fig. 2). Higher final competitive ability **A)** lowers final density in treatments without competition or disease, has no impact in treatments with only **B)** competition or **C)** disease, and **D)** increases it in treatments with both competition and disease. Key to abbreviations: *C* = competitor/diluters; *P* = parasites; *V* = standing trait variation. Colors follow Fig. 2.

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EDUCATION

Dec. 2016	Ph.D. in Ecology, Evolution, and Behavior, Department of Biology, Indiana University, Bloomington, Indiana, Advisor: Spencer Hall
May 2011	A.B. in Environmental Studies, Biology/Ecology Track, Minors in Biology & Music, Washington University in Saint Louis

APPOINTMENTS

Spring ’17	Postdoctoral Fellow, University of Minnesota (Advisors: Elizabeth Borer, Eric Seabloom, & Allison Shaw)
Spring ’17	Postdoctoral Fellow, Indiana University (Advisor: Spencer Hall)
Fall ’16	Final Year Fellow, Indiana University (Floyd/Ogg/Cleland Award)
Fall ‘12-Spr. ‘16	NSF GRFP Fellow
Fall ‘08-Spr. ‘11	Lab Assistant, Chase Lab, Washington University (P.I. Jon Chase)

PUBLICATIONS

Strauss, A.T., Shocket, M.S., Hite, J. L., Duffy, M.A., Cáceres, C.E., and S.R. Hall. *In prep*. Rapid evolution buffers densities of hosts during epidemics and maintains the dilution effect.

Strauss, A.T., Bowling, A.M., Duffy, M.A., Cáceres, C.E., and S.R. Hall. *In prep*. When and how diluters reduce disease: Host traits predict experimental outcomes of friendly competition.

Strauss, A.T., Shocket, M.S., Civitello, D.J., Hite, J.L., Penczykowski, R.M., Duffy, M.A., Caceres, C.E., and S.R. Hall. 2016. Habitat, predators, and hosts regulate disease in Daphnia through direct and indirect pathways. *Ecological Monographs* 86: 393-411.

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***Strauss, A.,** Civitello, D.J., Cáceres, C.E., and S.R. Hall. 2015. Success, failure, and ambiguity of the dilution effect among competitors. *Ecology Letters* 18: 916-926.

Strauss, A. and K.G. Smith. 2013. Why does amphibian chytrid (*Batrachochytrium dendrobatidis*) not occur everywhere? An exploratory study in Missouri ponds. *PLOS ONE.*

Strauss, A., White, A., and M. Boots. 2012. Invading with biological weapons: The importance of disease-mediated invasions. *Functional Ecology* 26: 1249-1261.

***** Outstanding Paper Award, ESA Disease Ecology Section, 2015

FELLOWSHIPS AND AWARDS

Spring 2016	Floyd/Ogg/Cleland Final Year Fellowship (IU)
Summer 2015	Outstanding Paper Award Recipient, ESA Disease Ecology Section
Spring 2015	Floyd Plant & Fungal Biology Summer Fellowship (IU)
Spring 2014	NSF Doctoral Dissertation Improvement Grant (DDIG)
Spring 2013	David G. Frey Memorial Fund Award (IU)
Spring 2012	Floyd Plant & Fungal Biology Summer Fellowship (IU)
Spring 2011	NSF Graduate Research Fellowship Program (GRFP)
Spring 2011	IU Department Research Recruitment Fellowship
Summer 2010	Tyson Undergraduate Research Fellowship (TURF)
Summer 2009	Summer Undergraduate Research Fellowship (SURF)

RESEARCH GRANTS

2014-2017	NSF DEB Pop. Comm. Ecology (helped write)	$376K awarded
2014-2015	NSF Doctoral Diss. Improvement Grant (DDIG)	$15K awarded
2013	David G. Frey Memorial Fund Award	$2K awarded

PRESENTATIONS

2016 ESA Talk: “Host traits and modular species interactions predict dynamical disease outcomes”

2015 ESA Talk: “Habitat, hosts, and fungus in the field: Synthesizing hypotheses from the community ecology of disease”
EEID Poster: “Integrating Community Drivers of Disease”
MEEC Talk: “Habitat, hosts, and fungus in the field: Synthesizing hypotheses from the community ecology of disease”

2014 ESA Talk: “The dilution effect among competing, evolving hosts”
*ESA, Presenter: Sarah A. Duple; Talk: “Intraspecific variation in interspecific competitive ability: Not all competitors are created equally”

2013 ESA Talk: “Outcomes of the dilution effect when hosts compete”
EEID Poster: “When hosts compete: Trait dependent outcomes of the dilution effect”

2012 ESA Poster: “Invertebrate community structure helps explain the distribution of amphibian chytrid in Eastern Missouri”

***** Talk presented by an undergraduate mentee

TEACHING & MENTORING

Pedagogy:

Spring 2015	Enrolled Course: “Mentored Teaching” (Prof. Mimi Zolan)

Teaching Positions:

Spring 2015	Associate Instructor, Honors Evolution, IU (Prof. Emília Martins)
Fall 2011	Associate Instructor, Bio 113, IU (Prof. Jim Hengeveld)

Spring 2011 Teaching Assistant, Intro to Ecology, Wash U
(Prof. Eleanor Pardini)

Invited Lectures:

Fall 2016 What do disease ecologists study?
How do ecologists study disease?
IU, Ecology; Bio 473 (Prof. Tara Darcy-Hall)

Spring 2015 Why do I have to take Calculus for my Biology major?
IU, Mentored Teaching (Prof. Mimi Zolan)

Spring 2015 Evolution in Disease Systems: How and why hosts and parasites evolve
IU, Honors Evolution; Bio S318 (Prof. Emilia Martins)

Fall 2014 Disease Ecology: Why and how do ecologists study parasites & disease?
IU, Ecology; Bio 473 (Prof. Tara Darcy-Hall)

Mentoring:

Undergraduate Research

Summer 2016 - present Mentee: Alison Partee
Fall 2015 – present Mentee: Andrew Sickbert

Groups Scholars STEM Initiative Mentoring (minority students); Indiana University

Fall 2013 – Spring 2015 Mentee: Johnathan Nguyen
Summer 2014 Mentee: Shawn Hall
Summer 2013 Mentee: Zephyr Wenrich

Jim Holland Summer Science Research Program (minority students); Indiana University

Summer 2014 Mentee: Sean Szolek-Van Valkenburgh

Biomath Fellows Program; U. Illinois (Director, Prof. Carla Cáceres and Zoi Rapti)

Summer 2013 Mentee: Sarah Duple

Undergraduate Honors Degree Individual Study (L490); Indiana University

Spring 2013 Mentee: Brad Lufkin

SERVICE & OUTREACH

Conference Organization:

Spring 2015 Midwest Ecology & Evolution Conference (MEEC), Indiana University Food, Posters, Venue, and Donations Committee

Journal Clubs:

Fall ‘15 – Spring ’16 Ecolunch Committee Member and Organizer
Spring ‘14 – Fall ‘14 Disease Journal Club Organizer

Outreach:

Fall ‘14 – Spring –‘15 Mystery Skulls Outreach; Indiana University & K-8 Schools (Director, Prof. Armin Mozcek)

Manuscript Reviewer:

Journal of Applied Ecology (1)